BACTERIOLOGICAL PROFILE OF PYODERMA IN PAEDIATRIC AGE GROUP (0-12YEARS) ATTENDING DERMATOLOGY OPD IN TERTIARY CARE HOSPITAL, SOUTH INDIA.

Dissertation submitted in

Partial fulfillment of the Regulations required for the award of

M.D. DEGREE

In

MICROBIOLOGY– BRANCH IV

The Tamil Nadu

DR. M.G.R. MEDICAL UNIVERSITY

Chennai

APRIL 2016.

CERTIFICATE

This is to certify that the enclosed work "**BACTERIOLIGICAL PROFILE OF PYODERMA IN PAEDIATRIC AGE GROUP (0-12 YEARS) ATTENDING DERMATOLOGY OPD IN TERTIARY CARE HOSPITAL, SOUTH INDIA"** submitted by **Dr.J.PANDIAN** to The TamilnaduDr.M.G.R. Medical University is based on bonafide cases studied and analyzed by the candidate in the Department of Microbiology, Coimbatore Medical College Hospital, during the period from July 2014 to June 2015 under the guidance and supervision of **Dr.V.SADHIQUA, DGO., M.D., Associate Professor** Department of Microbiology.

Guide

Dr.V.SADHIQUA, DGO., M.D,
Associate Professor,
Department of Microbiology
Coimbatore-14

Dr.A,EDWIN JOE, M.D(FM).,BL.
DEAN,
Coimbatore Medical College and Hospital
Coimbatore – 14.

Dr.A.DHANASEKARAN, M.D.,DCH
Professor and HOD,
Department of Microbiology
Coimbatore Medical College,
Coimbatore – 14.

DECLARATION

I, Dr J.PANDIAN solemnly declare that the dissertation entitled **"BACTERIOLOGICAL PROFILE OF PYODERMA IN PAEDIATRIC AGE GROUP (0 - 12 YEARS) ATTENDING DERMATOLOGY OPD IN TERTIARY CARE HOSPITAL, SOUTH INDIA"** was done by me at Coimbatore Medical College Hospital, during the period from July 2014 to June 2015 under the guidance and supervision of **Dr.V.SADHIQUA, DGO., M.D.,** Associate Professor, Department of Microbiology, Coimbatore Medical College, Coimbatore.

This dissertation is submitted to The TamilnaduDr.M.G.R. Medical University towards the partial fulfillment of the requirement for the award of M.D Degree (Branch - IV) in Microbiology.

I have not submitted this dissertation on any occasion to any University for the award of any degree.

Place:

Date: **Dr.J.Pandian**

Coimbatore Medical College

COIMBATORE, TAMILNADU, INDIA - 641 014

(Affiliated to The Tamilnadu Dr. MGR Medical University, Chennai)

ETHICS COMMITTEE

CERTIFICATE

Name of the Candidate : Dr. J. Pandian

Course : M.D. Microbiology

Period of Study : 2013 - 2016

College : Coimbatore Medical College

Dissertation Topic : Bacteriological Profile of PYODERMA in Paediatric age group (0-12 yrs) attending dermatology OPD in tertiary care hospital, south india.

The Ethics Committee, Coimbatore Medical College has decided to inform that your Dissertation Proposal is accepted / ~~Not accepted~~ and you are permitted / ~~Not permitted~~ to proceed with the above Study.

DEAN
Coimbatore Medical College & Hospital,
Coimbatore

15.7.2014

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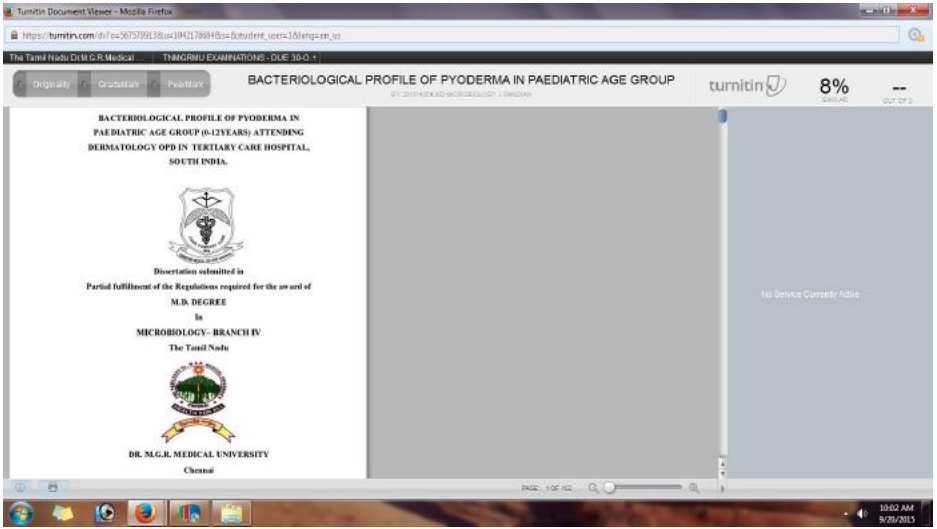
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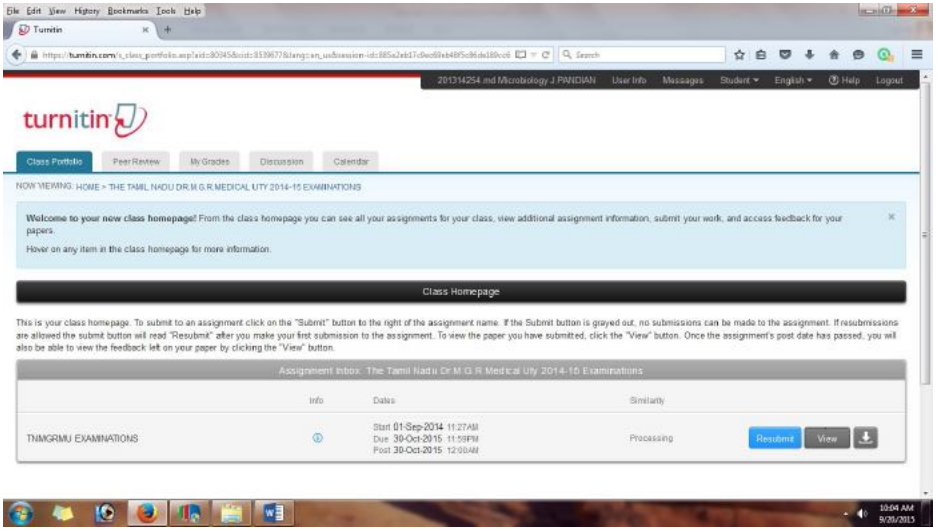
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LIST OF ABBREVIATIONS

HA-MRSA	Hospital acquired methicillin resistant staphylococcus aureus
CA-MRSA	Community acquired methicillin resistant staphylococcus aureus
S.pyogenes	Streptococcus pyogenes
S.aureus	Staphylococcus aureus
CoNS	Coagulase negative Staphylococcus
OPD	Outpatient department
IP	In-patient
MSSA	Methicillin sensitive staphylococcus aureus
VISA	Vancomycin intermediate Staphylococcus aureus
VRSA	Vancomycin resistant staphylococcus aureus
MHA	Muller Hinton Agar
ATCC	American type culture collection
MIC	Minimum Inhibitory Concentration
Pvl	Panton-Valentine Leukocidin
MuH	Mupirocinhigh level resistance
SCC	Staphylococcal chromosome cassette

INTRODUCTION

INTRODUCTION

Skin is an organ of utmost importance for human beings and also the largest and thinnest organ in humans[1]. It serves as a protective shield between the internal organs and the external environment[1]. Skin is one of the vital structures of human body is that in performs an unique role in the control of body temperature, excretion of water and salts, and also serves as an important sensory organ[1]. Skin when intact resists colonization by pathogenic microorganisms[2].

Anatomically skin is divided into three distinct layers-epidermis, dermis and subcutaneous tissue[1]. Skin is separated from deep structures by a layer of striated muscle[3]. The **epidermis** forms the first and outer most layer, mainly made of squamous epithelium and contains hair follicles, sebaceous glands and sweat glands[3]. The dermis forms the middle layer composed of dense connective tissues and is rich in blood supply and nerve endings[3]. The inner layer which comprises the **subcutaneous tissue** is composed of loose connective tissue and subcutaneous fat[3].

Skin acts as an important organ of protection by blocking ultra violet rays which affects epidermal nuclear layer and the dermis, by constant immune surveillance by Langerhans cells, pH and chemical defences by creating high salt and acidic environment, and all these properties prevent skin from colonisation by pathogens[3]. The outermost layer of skin-epidermis is composed of cells containing keratin a water repellent protein[3]. The normal microbial flora of the skin comprises certain bacteria like Diptheroids, Coagulase negative staphylococci (CoNS) and

Propionibacterium acnes[1]. CoNS are inoculated during vaginal passage and Coryneform bacterias take up residence on neonatal skin shortly after birth, the microbiome of neonatal skin includes many species of bacteria and fungi[2].

Superficial layer of skin is stratum corneum, this layer protects skin from both skin commensals and pathogenic microorganisms[3]. Neonates and children are easily susceptible to pathogenic bacteria since they have minimal protective cutaneous flora[4]. Bacterial skin infections can arise from invasion of microorganism from the external environment or through disruption in surface integrity of the skin or from the organisms that reach the skin via the blood stream as part of systemic disease[5]. However children are exposed to various types of trauma to the skin by insect bites, thorn pricks, abrasions, lacerations and due to burns[6]. These conditions provide opportunity for entry of microorganisms into various layers of the skin[6].

The skin lesions caused by pathogenic bacteria is prevalent worldwide, it is more common in developing than developed countries[7]. And they are more commonly seen during hot, humid climate and more common among low socioeconomic population, due to overcrowding and poor hygiene[8].

Skin infections are a major health problem among paediatric age group[8]. Around 25% to 30% of patients attending skin out- patient department belong to paediatric age (0-12 years)[8]. The prevalence of skin infection among children in various parts of India ranges from a minimum of 8.7% to a maximum of 35% and most of the children are below the

age of 10 years[8]. The skin of a new born child is sterile but on first exposure to the environment, the neonate skin is colonised by a large number of bacteria that are acquired from the surrounding environment[2].

Skin infections often manifest as macules, papules, nodules, pustules, vesicles and bullae etc., and are divided based on the layer in which infection has occurred[1]. Infection of epidermis and dermis are Impetigo, ecthyma, folliculitis, furuncles, carbuncle and infection of subcutaneous tissues are abscess, ulcers, boils[3]. Impetigo is the most common bacterial skin infection among children and is a superficial infection of skin initially vesicular which later becomes crusted[3]. It occurs as superficial, intra epidermal, unilocular and pustular lesions usually on exposed areas of body like face and leg[3].

Pyoderma are purulent infections of the skin and its appendages caused by pyogenic bacteria[6]. If untreated, pyoderma can extend to dermis resulting in ecthyma or furuncle formation[4]. Skin and soft tissue infections are classified based on the morphological and clinical picture into primary and secondary pyoderma[9]. **Primary pyoderma** is pyogenic infection of normal healthy skin due to disruption in the integrity of the epidermis, such as Impetigo, folliculitis, furunculosis, carbuncle, ecthyma etc[9]. **Secondary pyoderma** are pyogenic infection in a previously diseased skin such as scabies, dermatitis, eczema etc[6].

Majority of pyodermal skin infections are due to Staphylococcus aureus 78% and Group A Beta haemolytic Streptococcus pyogenes 8%[10].

Less common organisms are Enterococci and rarely Gram negative bacilli such as E.coli, Klebsiella, and Pseudomonas[10].

Usually a single pathogen is isolated in Pyoderma because Staphylococci produce bacteriocins which is highly bactericidal to Group A Streptococcus[6]. At times more than one organism are encountered (polymicrobial)[10]. Staphylococcus aureus is not part of normal skin flora but colonises the skin transiently or permanently in certain sites such as nose, axilla, groin, perineum[3]. Hence it causes significant opportunistic infections, under certain conditions like defects in leucocyte chemotaxis either congenital or acquired, immune compromised conditions like juvenile diabetes mellitus, malignancy, and congenital heart diseases[11]. Staphylococcus aureus colonization sites in children varies from that of adults and they usually get colonized in skin, umbilicus, and conjunctivae and fore skin of penis[3].

Streptococcus pyogenes does not colonise normal skin because surface lipids especially free fatty acids inhibits its growth[11]. Group A streptococcus infection is spread by transfer of organism from an infected person through close contact[12]. Impetigo caused by Group A Streptococcus occurs predominantly in pre-school children below 2 years of age whereas Group B, C, G may also be responsible for rare cases of impetigo[10]. Streptococcal skin infections if recurrent and untreated can cause serious complications like Post Streptococcal glomerulonephritis which increases the morbidity particularly in developing countries[12].

The prompt treatment of pyodermal skin infections includes early identification of risk factors for specific pathogens and early initiation of antimicrobial therapy after culture and sensitivity reports rather than empirical treatment[13].

The problem of emergence of drug resistant strains is increasing, probably due to indiscriminate and injudicious use of antibiotics[13]. Further the trend of etiological agents in different types of pyoderma is changing[6]. Methicillin resistant Staphylococcus aureus (MRSA) strains emerged in late 1960[11]. Initially MRSA strains were isolated as nosocomial pathogen[14]. These MRSA strains pose a major threat to the community because treating them remains a challenge for medical institutions[14]. The MRSA strains are divided into Hospital acquired MRSA (HA-MRSA) and community acquired MRSA (CA-MRSA)[14]. According to CDC, CA-MRSA is defined as MRSA culture positivity in out-patient setting or within 48 hours of hospitalisation in patient who had no history of risk factors for MRSA, like hospitalisation, surgery, indwelling devices, and dialysis[15]. CA-MRSA strains usually cause skin and soft tissue infections[15]. Sometimes they may cause serious infections like pneumonia and necrotizing fasciitis[15]. The clinical, molecular and antibiotic sensitivity patterns of CA-MRSA are different from HA-MRSA[14].

First case of CA-MRSA was reported in a children in 1988[16]. In India first PVL producing CA-MRSA strain was reported in 2009 from a child in Chennai[15]. Initially CA-MRSA infections reflected certain

circumstances like intra venous antibiotic use, treating out patients with reserved drugs[14]. At present CA-MRSA infections are now being encountered in community and population who do not reflect these risk factors or behaviour[15]. In 1996 Vancomycin intermediate staphylococcus aureus (VISA) was reported in Japan[11].

The emergence of CA-MRSA in paediatric age group is of great concern[15]. Attempts to eradicate MRSA has been done with mupirocin and indiscriminate use of this topical antibiotics led to emergence of mupirocin resistance among these strains[14].

Recent studies in India has confirmed the prevalence of CA-MRSA, but the percentage of incidence varies in different areas[17]. More cases have been reported in our country from south rather than from the northern region[18].

In this context our study was conducted to determine the bacteriological profile and Antibiotic sensitivity pattern (AST), in different types of pyoderma and the changing patterns of antibiotic sensitivity over a period of time among paediatric age group visiting Dermatology OPD department in CMCH.

AIM & OBJECTIVES

AIM AND OBJECTIVES

AIM:

To study the causative organisms and their current antibiotic susceptibility patterns in pyoderma cases, especially in children less than 12 years of age.

OBJECTIVES:

1) To study the bacteriological profile of pyoderma cases in paediatric age group.
2) To assess the antibiotic sensitivity pattern of the isolates.
3) To determine the resistance pattern among the isolates.
4) Genotyping of the resistant strains .
5) To identify CA-MRSA strains among Staphylococcus aureus isolates and their prevalence in skin and soft tissue infections in children.

REVIEW OF LITERATURE

REVIEW OF LITERATURE

Skin the largest and heaviest organ of our body is an extraordinary structure[19]. The surface area of skin is 1.7 m^2 length[20] .The structural make-up of the skin is similar in the limbs and trunk and differs from that of palms, sole and face[20]. The scalp and genital skin is unique by their morphology and function[4].

Human skin is made up of three layers epidermis, dermis and subcutaneous tissue or subcutaneous fat[3]. Epidermis is the outermost layer of skin and made up of keratinocytes, or epidermal cells[3]. Second layer is dermis composed primarily of collagen and adnexal structures like hair follicles, sebaceous glands, apocrine glands, eccrine glands and numerous blood vessels, lymphatics and nerves[3]. Third layer beneath the dermis is the subcutaneous tissue which consists of adipose tissue, large blood vessels and nerves[4]. In addition it has base of hair follicles and sweat glands[4].

The outer layer epidermis has four layers, the basal cell layer, spiny cell layer, granular layer and cornified layer[4]. The basal cell layer (stratum basalis) is made of columnar or cuboidal cells which are linked with basement membrane, a structure that separates epidermis from dermis[3]. Stratum basalis layer contains germinative cells and mitosis occurs in this layer .Above the basal layer is the spiny cell layer (stratum spinosum) composed mainly of desmosomes and keratin filaments that gives the cells a characteristic spiny appearance[3]. Above this layer is the granular cell layer (stratum granulosam)[3]. It consists of kerotohyalin granules which

gives this layer a characteristic granular appearance[3]. The superficial layer of the epidermis is cornified layer (stratum corneum) also called the horny cell layer[3]. The major cellular component of epidermis is keratinocyte mainly located in the spiny cell layer[3]. These cells as they move towards skin surface lose its organelles and nuclei to become elongated flattened cell called corneocytes[3]. Keratinocytes produce a filamentous protein called keratin which is present in skin, hairs and nails and plays an active role in the immune function of skin[4]. Stratum corneum acts as an important cutaneous barrier in protecting the body surface from toxins, desiccation and ultra violet rays which damage the living cells[3].

In addition to keratinocytes, the other cells in the epidermis are melanocytes, Langerhans cell and Merkel cells[3] .Melanocytes are most common dentritic cell seen in the basal layer and their main function is to synthesise and secrete melanin. Langerhans cell are bone marrow derived cells of monocyte macrophage lineage distributed mainly in stratum spinosum layer[3]. These cells internalize external antigens and process these antigens for presentation to T- lymphocytes in lymph nodes[3]. So they act as antigen presenting cell and has very important immune surveillance functions. Merkel cells are small in numbers and are frequently in contact with nerve fibrils[3].

Basement membrane zone appears as a homogenous band measuring 0.5 to 1.0 mm thick and is an important structure that attaches the basal

cell layer of epidermis to dermis and functions mainly in maintaining skin integrity[3].

Dermis is the middle layer and its thickness varies in different sites in our body[4]. Dermis is 30-40 times thicker than epidermis over the back of trunk. Dermis is organised into two distinct areas papillary dermis and reticular dermis. The superficial papillary dermis is a relatively thin zone beneath the epidermis and made of thin delicate collagen fibres and is highly vascularised[19]. The hair follicles are enveloped by a perifollicular dermis. The deeper part of dermis is the reticular dermis and this part forms the bulk of dermis[3]. It is less vascular than papillary dermis and has thick well organised collagen bundles[3].

Dermis contains adnexal structures like sebaceous, apocrine, eccrine glands and hair follicles[3]. Sebaceous gland as part of the pilosebaceous unit its function is production of sebum[3]. Sebum travels through the sebaceous duct to reach the hair follicle and covers the skin surface and serves like a protectant. Sebum also has antifungal property[4]. Sebaceous glands are located everywhere on the skin except the palms and soles[3]. Eccrine glands are embryologically derived from epidermis and are not part of the pilosebaceous unit[3].

The main function of the eccrine sweat glands is temperature regulation via secretion of sweat which is a combination of water and electrolytes[3]. The ducts of the sweat gland pass through dermis and epidermis to empty the sweat onto the skin surface.As sweat evaporates it

cools the skin surface[4]. This eccrine glands are located everywhere on the skin surface except on modified areas such as lips, nail beds and glans penis[3] .Apocrine glands arise from the same hair germ that gives rise to hair follicle and sebaceous gland[4]. The apocrine gland empties into the follicle above the sebaceous gland, the main function of which is to produce smell and these glands are primarily located in the axillae and perineum. Their activity is sex hormone dependant. The breast and cerumen glands are both modified apocrine sweat glands[3].

The main cellular component of the dermis is fibroblast. The fibroblast produces the main components of the dermis mainly collagen (70-80%), elastin (1-3%) for elasticity and proteoglycans to maintain water within the dermis[4]. The collagen serves as the major structural protein of the entire body and represents 70% of dry weight of skin and responsible for maintenance of elasticity of skin[4]. The bulk of the collagen within the dermis consists of type1 and type3 collagen and is organized into collagen bundles that run horizontally through dermis[3].

The main function of dermis is maintenance of normal body temperature, which is regulated by control of blood flow and sweating, by the dermal vessels and eccrine glands[3]. Dermal vasculature is superficial and deep plexus of arterioles and venules that are interconnected by communicating vessels[3]. Lowering body temperature is accomplished through increased blood flow in the vascular plexus in the high papillary dermis, allowing heat to be removed through radiation from the skin[3].

Another function of dermis is mechanical protection of underlying structures, achieved primarily by the collagen and hyaluronic acid. Innervation of skin mostly occurs in the dermis and is responsible for cutaneous sensation[3].

Subcutaneous tissue or subcutaneous fat is the inner layer of skin and lies beneath the dermis. Subcutis are divided by fibrous septae which is composed of collagen[4]. This layer is abundantly present over abdomen and buttocks and functions as a caloric reserve. It also serves as a heat insulator and shock absorber and also as an endocrine organ. The conversion of androstenidione to estrone by aromatase takes place in subcutaneous tissue[4]. Leptin a hormone that regulates body weight is produced by lipocytes .Important metabolic products like vitamin D3 are synthesised in skin. Blood vessels, nerves and lymphatics are also found in fibrous septae[3].

NORMAL FLORA OF THE SKIN:

The population of microorganisms that inhabit many skin surfaces of healthy human body are termed as normal flora or normal microbiota[2].

Skin the largest and sensitive organ in human body has diverse normal microbiota in its surface and they provide the first line of defense against pathogenic microorganisms[2]. It also plays a major role in toxin degradation and in maturation of the immune system by skin associated lymphoid tissue[1]. The skin has many protective properties. The outermost

acellular layer of skin along with tightly packed cellular layer acts as a physical barrier by preventing entry of microorganisms[3]. Human skin has the property of shedding or sloughing of outer layer continuously, so that the pathogenic bacteria are continuously dislodged from the skin surface[3]. Dry, acidic, cool environment usually prevails in skin which is not suitable for growth of bacteria which prefer warm, moist climate for survival and growth. The hair follicles, sweat glands, sebaceous glands of skin produce natural antibacterial substance, acidic substance (low pH), toxic lipids, alcohols and sebum, which limits bacterial growth[3].

Skin contains diverse array of microorganisms and they are categorised as resident and transient microbiota[2]. Resident microbiota are fixed type of organisms that are non- pathogenic and usually present in specific areas and re-establishes itself if disturbed[2]. Transient microbiota are a mixture of both potential pathogenic and non- pathogenic group, these organisms are mostly seen in environment and do not produce disease as they do not reside permanently on skin surface[2]. Transient microbiota behaves as non-pathogenic when normal resident flora are intact, but in extreme conditions when resident flora is disturbed this transient microbial flora invade the area and multiply to behave like pathogens and cause disease[1].

The normal bacterial population present in human skin varies with anatomic location, environmental conditions like temperature and presence of oxygen[1]. The normal microbiota prefer moist areas of body and their

favoured sites are hair follicles, sebaceous glands and sweat glands, skin folds, under arms, the genitals or areas around anus and skin around mouth, face and scalp surface[1].

The normal flora of skin in children are different from adult population[3]. The dominant resident microbiota frequently isolated from many areas of human skin are aerobic and anaerobic diptheroid bacilli like Corynebacterium, Propionibacterium acnes and nonhemolytic aerobic and anaerobic Staphylococci mostly coagulase negative staphylococcal species and Micrococcus species[1].

Rarely Staphylococcus aureus and Peptostreptococcus species, Gram positive aerobic spore forming bacilli that are present in air, water and soil, alpha haemolytic and non-haemolytic Streptococcus (viridans and mitis streptococci) and Acinetobacter are sometimes encountered[2]. Occasionally Fungi and yeasts are seen residing in skin folds mostly Candida species[2]. Sometimes Nonpathogenic mycobacteria are isolated from genitalia and external ear as these areas are rich in sebaceous secretions which helps in their growth[2]. The resident microbiota acts by protecting skin surface from pathogen invasion by competing for recptors or binding sites in host cell and by competing for nutrients this mechanism of protecting skin surface is called bacterial interference[2]. Other factors which help in eliminating non-resident microorganism from skin surface are low pH , fatty acids and presence of lysozyme .Sometimes the normal resident flora may be affected by daily vigorous scrubbing with alkaline soap which makes the skin

surface dry and raises the pH[2]. But normal resident flora is replaced immediately by microorganisms present in sebaceous and sweat glands[2].

The New born encounters microbial agents during delivery process, the baby travels from sterile environment of mother's uterus through the birth canal which is heavily colonized by microbial agents[2]. The skin of the new born gets colonized by microbiota within 5 minutes after delivery[2]. Baby is delivered by normal vaginal delivery are usually colonized predominantly by Lactobacillus, Prevotella, Atopobium species of the birth canal. Babies delivered by caesarean section have microbiota similar to the skin microbiota of the mother[2], commonly by staphylococcus, Corynebacterium and Propionibacterium Species[2].

Human Skin behaves as an efficient physical and chemical barrier and apart from this it also acts as an immunologic barrier by its specialised cellular network. Keratinocytes the important principle cell continuously monitors the microbial flora that is present over surface of skin, through pattern recognition receptors such as toll like receptors, mannose receptors, and nod like receptors[2]. When pathogens invade skin surface the recognition receptors initiate the immune response, and start secretion of antimicrobial peptide, cytokines and chemokines which act promptly in controlling the pathogens that try to colonize the skin surface[2].

MANIFESTATION OF SKIN LESIONS:

Morphology of skin lesions visible on the surface of the skin can provide clue for identification of infection and probable causative agents. Skin lesions are classified as primary, secondary and special skin lesions[3].

Primary skin lesions that are not been altered by trauma, manipulation (scratching, scrubbing) or natural regression over time. Examples of primary lesion include macule, wheal, papule, vesicles, plaques, bullae, patches, pustules, nodules, cysts[3].

A macule is defined as a flat, circumscribed skin discolouration that lacks surface elevation or depression mostly caused by Dermatophytes, Treponema pallidum(secondary syphilis) and Enterovirus (exanthemum rash) infections[1]. Papule is an elevated solid lesion less than 0.5 cm in diameter mostly encountered in Staphylococcal, Pseudomonal and Sarcoptes scabiei infections, Human pappilomavirus types3,10 and Pox virus (molluscum contagiosum)[1].Patch is a flat, circumscribed skin discolouration or a very large macule[3].

Nodule is defined as elevated, solid lesion more than 0.5 cm in diameter, this is a larger, deeper papule often seen in Staphylococcus aureus, Nocardia, Corynebacterium diptheriae and Mycobacterium marinum infections[1]. Vesicle is a circumscribed, raised, fluid filled (blister-like) lesion less than 0.5 cm in diameter often seen in virus infections[1].

Bulla is a localised, raised, fluid filled lesion more than 0.5 cm diameter seen as a large vesicle and seen in Staphylococcus aureus(bullous

impetigo and scaled skin syndrome), Clostridial species(necrotizing gas gangrene),Herpes simplex virus and other skin infections[1]. Wheal is usually seen as a firm, edematous plaque that is evanescent and pruritic as seen in utricaria demographism[3]. Pustule manifests as papule that contains purulent material, mostly seen in folliculitis, impetigo[1]. Cyst is a nodule that contains fluid or semisolid material frequently associated with acne, epidermal inclusions[3].

Secondary skin lesions are created by scratching, scrubbing or secondary infection and these lesions develop normally in time. Secondary lesion include crusts, scales, ulcers, fissures, excoriations, scar, erosions and post inflammatory hyperpigmentation[3]. Crusts are defined as a collection of cellular debris, dried serum and blood, a scab is an antecedent of primary lesion usually a vesicle, bulla or pustule. Erosions are partial focal loss of epidermis which heals without scarring[3]. An Ulcer is defined as a full thickness ,focal loss of epidermis and dermis which heals with scarring, usually see in lesion of Bacillus anthracis (cutaneous anthrax), Haemophilus ducreyi(choncroid), Treponema pallidum(chancre of primary syphilis) and bowel flora(decubiti)[1].Excoriations are linear erosions induced by scratching usually observed in scabies and insect bite allergy lesion[3].

Special skin lesions are burrows, comedomes, petechiae, purpura. Burrow is an elevated channel present in superficial epidermis produced mainly by a parasite such as mite Sarcoptes scabiei and mostly seen over

wrists and finger web spaces. Come- domes are folliculocentric collection of sebum and keratin usually called acne[3].

PYODERMAS:

The term Pyodermas is a collective term which refers to the infection of skin layers and its adnexal structures the hair follicle, sebaceous gland, apocrine gland and eccrine glands by pathogenic pus producing bacteria. 30% of patients visiting dermatologist are from paediatric age group[21]. Pyoderma cases account for 15%-20% of out-patients attending Dermatology department[21]. This skin problem is prevalent in all age – groups. However paediatric age group are the most affected[21]. It affects both gender and are seen in high intensity during hot and humid climate mostly in tropical countries[12]. Apart from seasonal variation poor hygiene, poor sanitation and malnutrition are the other important factors which play a major role in acquisition of pyoderma [5].Although this problem is seen worldwide it is more common in under developed and developing country than developed nations where the standard of living is better[7].

Devendra Mohan Thappa et al(2002) study on Common Skin Problems has shown that 30% of out patients visiting Dermatologist belong to Paediatric age[21].

Neirita Hazarika et al (2012) study shows that pyoderma is more prevalent in over- crowded, malnourished children[5].

Pyodermas are classified on the basis of morphological and clinical grounds which provides clue about the causative agents responsible for the lesion, into primary and secondary pyodermas[9]. The primary pyoderma arise in the normal skin surface and have characteristic morphology and usually the causative agent is a single pathogenic microorganism[22]. Secondary pyoderma arise from already diseased or damaged skin and these lesions lack specific morphology because here the primary lesion is invaded by pyogenic bacteria and usually more than one bacteria(polymicrobial) are isolated[9].

Study by Naresh Jain et al (2010) shows Pyoderma as the most common skin disease among paediatric age group[23].

PRIMARY PYODERMA	SECONDARY PYODERMA
Impetigo	Traumatic lesions (abarasions, insect bites, animal bites)
Folliculitis	Burns
Furuncles and carbuncles	Eczematous dermatitis and Scabies with secondary infection.
Ecthyma	Chronic ulcers
Erysipelas	Intertrigo
Paronychia	Pilonidal and sebaceous cyst
Cellulitis	Pyoderma gangrenosa
Membranous ulcers	Hidradenitis suppurativa
Chancriform ulcers	Vesicular eruptions (varicella, pemphigus)[14]

James Hedrick et al (2003) study states all common acute bacterial infections encountered in paediatric age population[13].

Impetigo:

Impetigo is the most commonly observed primary pyoderma. It is a highly communicable infection mostly among paediatric age group particularly in preschool children[5].

Study by Shashi Gandhi et al (2012) shows Impetigo constituted a major skin lesion among primary pyoderma[7].

This infection spreads fast in overcrowded and poor sanitation conditions[12]. They evolve as superficial infection of skin which appears to be vesicular initially and later become crusted[3]. These lesions are seen as superficial, intraepidermal, unilocular vesicles and pustules according to histopathological finding[3]. Children are the main victims and this condition is more prevalent in summer season[12].

The etiological agents isolated from impetigo lesions have changed over periods, initially group A streptococcus pyogenes which were the major causative agent followed by staphylococcus aureus to some extent[24]. Now the trend has been entirely reversed with staphylococcus aureus as the dominant agent in 70% -80% of cases and group A streptococcus in less than 20% of cases[5]. Staphylococcus aureus is found in the nose of many healthy individuals[25]. Staphylococcus aureus appears

to be the common secondary invaders of skin surface, because certain strains produce bacteriocins a chemical substance which is toxic to other bacterias[6].

Group A streptococcus pyogenes invades skin surface of healthy individual and remain dormant without causing any lesion even for about 10 days[14]. When the skin surface is damaged due to minor traumatic lesions then streptococci invade the skin and produce lesions which when left untreated for a period of two to three weeks lead to acute glomerulonephritis[11]. Impetigo starts as a small red macule with tiny, pinhead sized clear vesicles which are asymmetrically scattered with erythematous surrounding area[14].

Two clinical variants of impetigo are bullous impetigo and classical or nonbullous impetigo[3]. Bullous impetigo occurs more common in new born and paediatric age group and amounts to 10% of all impetigo[14]. It is mainly caused by staphylococcus aureus of phage group ll (type 71)[14].The lesions are clear large vesicles without any erythematous surrounding initially,later becoming flaccid bullae containing cloudy pus and finally the roof of bullae ruptures leaving a moist red surface covered with thin varnish like brown crusts[14]. The pathogenic staphylococcus aureus(phage group ll) which cause bullous lesions produce two types of extracellular exfoliative toxins A and B[11]. Exfoliative toxin A is chromosomaly encoded and the exfoliative toxin B is heat-labile and plasmid coded[11]. These toxins are glutamate specific serine proteases which

helps to bind and cleave desmoglein-1 a transmembrane protein necessary for epidermal cell adhesion, which results in splitting of desmosomes and results in blister formation at the site of infection[14]. Staphylococcus aureus of phage group ll is the only bacteria regularly isolated in this lesions because it produces bacteriocins which inhibits streptococci and other staphylococcal species[14].

Non bullous impetigo or classical impetigo is a communicable infection usually seen in overcrowded population and affects preschool children with poor hygiene[12]. This lesion is caused by both staphylococcus aureus and group A beta haemolytic streptococcus pyogenes[14]. Non bullous impetigo commonly arise in exposed areas of skin as small vesicles that becomes filled with pus rapidly and ruptures , the purulent discharge from the lesion forming a characteristic thick golden yellow crusts[3]. Streptococcus pyogenes which causes impetigo belong to M serotypes (2,49,52,55,57,59,60,61).Group B streptococci has also been isolated from impetigo in newborn[14] .

The important laboratory findings in diagnosing impetigo are Gram stained smears usually the exudate beneath an unroofed crust is the ideal specimen. The anti-streptolysin O titer gives false result as streptolysin O get destroyed by lipids present in skin. The anti-DNase B response is usually seen after streptococcal impetigo[11].

FOLLICULITIS:

Folliculitis is a minor infection of hair follicles and apocrine gland[4]. The lesions are small 2 to 5 mm pruritic papules topped by a central pustule pierced by a hair and surrounded by redness[3]. Folliculitis is seen frequently in scalp, face, neck and buttocks and not seen in areas such as palms and soles which lack hair follicle[19]. When situated deep inside the follicle they often destroy the wall of the follicle and spread to adjacent structures and manifest either as superficial or deep folliculitis[4].

Janardhanan.B et al (2015) study shows Folliculitis as the second most common lesion after Impetigo[26].

Superficial folliculitis is otherwise called as Bockhart impetigo[19]. This type of lesion is seen in humid climate and affects scalp of children and neck, face and axillae of adults[25]. The causative agent is staphylococcus aureus[14]. The lesions appear as small tense pustules with yellow dome shaped lesions, surrounded with erythema, after which they burst and crusts are formed in the follicular opening the lesion is self-limited[4].

Deep folliculitis otherwise called folliculitis simplex is usually caused by staphylococcus aureus and involves the entire hair follicle[4]. Multiple recurrent lesions are present and it is transmitted within family members[4].

There are several clinical variants of deep folliculitis namely folliculitis simplex barbae, folliculitis eczematosa, folliculitis eczematosa vestibule nasi and tufted hair folliculitis[19]. Sycosis barbae is a chronic folliculitis that occurs in bearded areas the causative agent being

staphylococcus aureus[4]. Pseudomonas aeruginosa serotype 0-11 is responsible for folliculitis acquired in swimming pools, hot tubs and whirlpools[14]. Folliculitis in perioral and per nasal areas are caused by enterobacteriaceae members[14]. Among the fungi candida may cause folliculitis by producing pruritic satellite lesions in intertrigenous areas in infants or in patients with prolonged antibiotic or corticosteroid therapy[4].

FURUNCLES AND CARBUNCLES:

The furuncle or boil is a tender deep inflammatory red nodule in a hair follicle that extends into subcutaneous tissue which has been developed from the preceding folliculitis[3]. The carbuncle is a more extensive furuncle that coalesce to involve multiple follicles that extends into the subcutaneous fat in areas covered by thick in elastic skin[3]. It later becomes organised as multiple abscesses which are separated by connective tissue septa and drain to the surface along hair follicles. The main causative agent is staphylococcus aureus[11].

The furuncles usually arise in hair follicles that are present in areas that are experiencing friction and perspiration[4]. The neck, face axillae and buttocks are common areas affected by furuncles[4]. Factors which enhance the formation of furuncles and carbuncles are obesity, patients on corticosteroid therapy, and defects in neutrophil function and diabetes mellitus[14]. A carbuncle usually arise in the nape of the neck, on the back and on thighs[14].

ECTHYMA:

The lesion of ecthyma appears similar to impetigo but penetrates through the epidermis, dermis and consists of punched out ulcers with dry hard adherent greenish yellow crusts surrounded by raised margins[3].It is usually seen as secondary infection in pre-existing superficial lesions like insect bites, excoriations[14]. Most common organism isolated in ecthyma is beta haemolytic group A streptococcus pyogenes[19]. It is seen more common in children and lesions develop on the exposed trauma prone sites. Glomerulonephritis is the main secondary complication[14].

ERYSIPELAS:

The erysipelas is also known as St Anthony's fire[4]. It is a distinctive type of superficial cellulitis of skin which involves the dermis and most superficial parts of subcutaneous tissue[4]. These lesion are swollen, painful, red and indurated with prominent lymphatic involvement (regional lymphadenopathy). It is always caused by Group A streptococcus pyogenes and sometimes by Group B , C or G streptococci[13].

Erysipelas is more common in infants, young children and elderly individuals and Group B streptococcal erysipelas is particularly seen in new born. Usually erysipelas lesions 70% to 80% involve the lower limbs and 5% to 20% are on the face[13]. The streptococcus gain entry via skin ulcers, local trauma or abrasions, psoriatic or eczematous lesions or fungal infections. But in new born the erysipelas develops from umbilical stump infection[14].

Erysipelas lesions are painful and bright red, oedematous, indurated (peau d orange) appearance and raised borders that is demarcated from adjacent normal skin. Most common area affected by erysipelas is the bridge of the nose and cheeks[3]. A butterfly pattern on the face is seen and there is a marked polmorphonuclear leucocytosis of 20000/mm3[14].

CELLULITIS:

Cellulitis is an acute diffuse, suppurative, spreading infection of skin that involves deeper layers of dermis and subcutaneous tissues, caused most frequently by group A streptococcus pyogenes or staphylococcus aureus and rarely by Aeromonas and Haemophilus influenza which typically affects young children[14].

In cellulitis, lymphangitis may spread from the area to the neighbouring lymph nodes and gangrene and severe sepsis may follow. In periorbital cellulitis the causative agents are staphylococcus aureus and streptococcus pyogenes whereas in postoperative wound infection streptococcus pyogenes is often isolated from wound pus[14].

NAIL FOLD INFECTIONS:

The main nail fold infections are paronychia, bulla repens, blistering dactylitis and felon. Paronychia are painful, erythematous and swelling of proximal nail fold but usually associated with an in grown nail or history of trauma, pus usually comes out from nail fold and crusts develop[14]. The most common organism isolated from cases of acute paronychia are staphylococcus aureus and candida albicans from chronic paronychia

lesions. Bulla repens is a staphylococcal infection which develops in thick acral skin in tip of digits[14]. Blistering dactylitis is a bullous eruption seen in tips of toes and fingers among paediatric age and is usually caused by group A beta haemolytic streptococci[14].

MEMBRANOUS ULCERS:

Membranous ulcers are ulcers in which the base is covered by a layer of necrotic tissue or membrane. The membrane is not adherent and can be removed easily and caused mainly by corynebacteium diptheriae[14].

SECONDARY PYODERMAS:

Many cutaneous lesions like insect bite, abrasion, wound, eczematous lesion ulcers and scabies are complicated by secondary or super imposed bacterial infection by masking the primary lesion[9]. Insect bite with secondary infection is most commonly encountered among secondary pyoderma followed by scabies with infection[6]. Staphylococcus aureus and group A streptococcus pyogenes are isolated from these pyodermal conditions[14].

Hidradenitis suppurativa important secondary pyoderma is a chronic disease of apocrine glands in axillary, genital and perianal areas. The primary lesion in this condition appears due to keratinous plugging of apocrine glands and ducts which latter leads to dilatation and rupture of the gland[14]. The lesions are tender, reddish purple nodules which become fluctuant and then ruptures. They are frequently infected secondarily by staphylococci, non- haemolytic streptococcal species, Escherichia coli,

Proteus and Pseudomonas and infrequently by anaerobic organism mainly Bacteroides species[14].

COMMON BACTERIAL SKIN INFECTIONS:

The most common organism isolated from pyodermal lesions in paediatric age group in community population are Staphylococcus aureus 70%-80% and followed by group A streptococcus pyogenes 8%-10% and rarely Enterococci , Escherichia coli, Klesiella and Pseudomonas[10].

Study by CRV Narasimhalu et al (2014) shows Common bacteria isolated from pyodermal lesions[27].

STAPHYLOCOCCAL INFECTIONS:

Staphylococci species produce a wide range of infections in humans and they are most frequently isolated from clinical samples[28]. Staphylococcus aureus is the most virulent organism among the staphylococcal species and remains a major cause of morbidity and mortality[28]. It is a pluripotent pathogen, causing disease by both toxin mediated and non-toxin mediated mechanisms[28]. Staphylococcus aureus is responsible for both nosocomial and community based infections of skin and soft tissues and also cause life threatening systemic infections[14]. The less virulent Staphylococci (Eg, S.epidermidis, S.sarophyticus, S.intermedius), collectively known as coagulase negative staphylococci (CoNS) are part of normal skin flora but in some conditions they behave as oppurtunistic pathogens[14].

Dr Sanjiv V Choudry et al (2013) study shows Staphylococcus aureus as common isolate from both Primary and Secondary Pyoderma[30].

Serious staphylococcus aureus infections are usually encountered by an individual in certain conditions such as defects in leukocyte chemotaxis which may be congenital(Wiskott-Aldrich syndrome, Down's syndrome, Jobs syndrome and Chediak-Highasi syndrome) and in Juvenile diabetes mellitus, defects in opsonisation or complement(C3 and C5), skin injuries like burns, abrasions, surgical incisions and eczema and also in chronic underlying conditions like malignancy and congenital heart diseases[11].

The genus staphylococcus belongs to family Micrococcaceae and contains 36 species[14]. It is an aerobe and facultative anaerobe, non-motile, Gram positive cocci about 1 µm arranged in clusters, single in pairs and tetrads. Staphylococcus aureus produces catalase and slowly ferments many sugars and forms lactic acid but not gas[30]. Each strain varies by its proteolytic activity and they produce many extracellular substances. Staphylococci can survive in dried items, tolerate heat (up to 50 degree for 30 minutes) and 9% sodium chloride[31].

VIRULENCE FACTORS OF STAPHYLOCOCCUS AUREUS:

I) Peptidoglycan and teichoic acids:

The cell wall of staphylococcus aureus contains peptidoglycan (cross linked polymers of N-acetyl-glucosamine and N-acetyl-muramic acid), and teichoic acids a unique ribitol (5- carbon monosacchride)-phosphate polymer[14]. The main function of teichoic acid is to help the bacteria

to adhere to mucosal surface and peptidoglycan provide rigidity to staphylococcal cell wall and contributes to virulence by activating complement, enhancement of chemotaxis of polymorph nuclear cells and interlukin-1 production by monocytes[11]. Other proteins present in peptidoglycan are adhesins, fibronectin binding proteins, collagen binding proteins and clumping factor[11].

Katarina Chiller et al's (2001) study shows Pathogenesis and virulence factors like Teichoic acid, Protein A involvement in S.aureus skin lesions[32].

II) Protein A:

Staphylococcus aureus cell wall contains specialized protein on its cell surface called protein A; they belong to group of adhesins called microbial surface components recognizing adhesive matrix molecules(MSCRAMMS)[32]. Protein A interferes with opsonisation and ingestion of organism by polymorphonuclear cells, activates complement and causes immediate and delayed type hypersensitivity reactions[32]. They are highly immunogenic and antibodies formed against protein A is detected in persons with fulminant staphylococcus aureus infection. Protein A binds to Fc fragment of IgG and the antigen binding site Fab is free to combine with any specific antigen and this property is called coagglutination[11].

III) Capsular polysaccharide:

Some strains of staphylococcus aureus produce exopolysaccharide that prevents them from ingestion by polymorphonuclear cells and there are 11 types of capsular polysaccharide[11].

IV) Enzymes:

Many enzymes are produced by staphylococcus aureus. The myeloperoxidase system present in phagocytic cells produce free radicals and toxic hydrogen peroxide which is toxic to ingested microorganism[11]. The enzyme catalase produced by staphylococcus aureus inactivates the free radicals and hydrogen peroxide and helps in its survival in the host. It has another cell bound material called clumping factor which helps in binding of fibrin and fibrinogen to their cell wall and safeguards them[11].

The enzyme coagulase exists in free and bound form and binds to prothrombin then becomes active and converts fibrinogen to fibrin and this fibrin forms a coat around the bacterial cells and make them resistant to opsonisation and phagocytosis[11].

Study by HK Tiwari et al (2008) shows Tube coagulase test has highest specificity (98.1%) and highest sensitivity of (98.7%)[33].

Enzyme fibrinolysin which is produced by staphylococcus acts by breaking the fibrin clots and enhances the spread of infection. Hyaluronidase hydrolyses the intracellular matrix in tissues and aids in the spread of organism in skin and subcutaneous tissues[11].

Phosphatidyl inositol specific phospholipase C damages the tissue by activating complement system[11]. Three different types of β-lactamase enzymes are produced by staphylococcus aureus these enzymes may be inducible (i.e., produced only in presence of β-lactam antibiotics) or constitutive (i.e., it is produced continuously), genes which codes for these enzymes are found on plasmids. These resistance genes may be transferred to other bacteria by transformation and transduction[11].

V) Hemolysins:

Four types of hemolysins are produced by staphylococcus aureus.α-hemolyin is a heterogenous protein which has lethal effect on polymorpho nuclear cells and erythrocytes.α-hemolysin acts as a dermonecrotic toxin and also as neurotoxin by causing demyelination of myelin sheath and this hemolysin is responsible for the hemolysis observed around colonies of staphylococcus aureus grown in sheep blood agar.β-hemolysin is a sphingomyelinase which acts on sphingomyelin present in many cells mainly human red blood cells, it is also known as hot- cold hemolysin[11].

Delta-hemolysin is produced by more than 97% of staphylococcus aureus strains, they act as surfactants and damage cell membrane. γ-hemolysin is made of 3 proteins this along with 2 proteins of Pantom valentine leukocidin(PVL) form six set of two component toxins, this hemolysin lyses leukocytes efficiently[14].

VI)Toxins:

Exfoliatins or epidermolytic toxins are produced by staphylococcus aureus,which are two different exfoliatins of same molecular weight[11]. Exfoliatin A (ET-A) is a thermostable protein for which the structural gene is chromosomal and exfoliatin B (ET-B) is heat labile and mainly of plasmid origin[11]. These toxins have proteolytic activity and dissolve the mucopolysaccharide matrix of the epidermis and results in intraepithelial splitting of cellular linkages in the stratum granulosam[14]. Some staphylococcal strains produce both the toxins simultaneously and cause staphylococcal scaled skin syndrome[11]. Some staphylococcus aureus strains which causes toxic shock syndrome, secrete a toxin called toxic shock syndrome toxin(TSST-1)[11]. Enterotoxins (A-E,H and I) are heat stable and are responsible for staphylococcal food poisoning[11].

Study by W M Johnson et al (1991) shows the ET-A, ET-B and Enterotoxins in Staphylococcus aureus[34].

VII) Superantigens:

The staphylococcal enterotoxins (A-E, G-j, K-r and U,V) along with toxic shock syndrome toxin(TSST-1) are collectively called pyrogenic toxin superantigens. They stimulate the proliferation of T lymphocytes without regard for their antigenic specificities[11].

SKIN INFECTIONS CAUSED BY STAPHYLOCOCCUS:

- Impetigo(bullous impetigo)
- Furuncles(boils)
- Carbuncles
- Superficial folliculitis(impetigo of Bockhart)
- Staphylococcal scaled skin syndrome
- Staphylococcal cellulitis
- Toxic shock syndrome
- Staphylococcal scarlet fever
- Wound infections
- Secondary infections of dermatitis.[28]

IDENTIFICATION TESTS:

I) Morphology in culture Medias:

Staphylococcus aureus grows well in simple media[30].

Nutrient agar- as smooth, low convex, glistening, densely opaque[30]. The pigment formation in nutrient agar when kept in room temperature in aerobic atmosphere ranges from cream colour to golden yellow due to presence of carotenoids[31].

Blood agar- as white opaque colonies surrounded by a zone of beta hemolysis[31].

Maconkey agar -they grow as small pink colonies[30].

Selective Medias-for isolating staphylococcus aureus are salt cooked meat broth, mannitol salt agar, chrom agar[11].

II) Biochemical characters:

Staphylococcus aureus produces catalase, ferments 10% mannitol to acid and 10%trehalose to acid. It also produces acetoin (Voges-Proskauer) in MR-VP broth after incubation for 48 hours[11]. And ferments glucose both aerobically and anaerobically in Modified Hugh and Leifson oxidation fermentation medium. Liquefies gelatin by enzyme gelatinase, produce deoxyribonuclease (DNase), produce heat stable nuclease thermonuclease (TNase) when grown in toluidine blue DNA agar producing a pink halo around the well. Indole negative, but urease is produced and gives positive reaction when grown in Christensen's urea medium[11].

The single most reliable test for identification of staphylococcus aureus is the coagulase test[33]. Slide coagulase test detects bound coagulase (clumping factor) present in S.aureus and absent in most other staphylococcal species[11]. Tube coagulase detects the presence of free coagulase which reacts with clot reacting factor (CRF) present in plasma, which in turn reacts with fibrinogen to form fibrin[30].

III) RAPIDEC staph:

It is a rapid test kit which detects the presence of enzyme aurease specific to coagulase positive staphylococci, the enzyme aurease reacts with prothrombin to form a complex that lyses the substrate to release a fluorogen which is detected by its fluorescence under ultraviolet radiation at 365 nm. Staphylococcus aureus is identified within 2 hours by this kit

method and also identifies MRSA strains that are not detected by conventional tests[31].

TREATMENT:

Staphylococcus aureus is frequently isolated from nose and skin of many individuals. Skin can be cleared of staphylococci and prevented from recurrent infection by use of local antiseptics. Abscess and closed suppurative staphylococcal lesions are treated by incision and drainage and antibiotics[28].

Few staphylococcus aureus strains less than 5% are susceptible to penicillin. But penicillin resistant strains are treated with penicillinase resistant drugs like oxacillin, methicillin and extended spectrum penicillins like ampicillin, amoxicillin and sometimes in combination with clavulinic acid. First generation cephalosporins are used frequently in treating staphylococcus aureus skin infections[28]. Macrolides like erythromycin, azithromycin and aminoglycosides gentamycin, amikacin are also used frequently and Quinolones ciprofloxacin, levofloxacin to some extent. Tetracycline analogue doxycycline is also used in treating staphylococcal skin infections in older children and adults. Few children with staphylococcus aureus skin infections who are not exposed to higher antibiotic respond to treatment by trimethoprim-sulfamethoxazole[28].

For methicillin resistant staphylococcus aureus skin infections glycopeptide antibiotic Vancomycin, teicoplanin and Oxazolidinone group

of drug linezolid is used. Fifth generation cephalosporin Ceftaroline also used when the strain is resistant to other drugs. Local application of fusidic acid, Mupirocin ointment are commonly used[28].

ANTIBIOTIC RESISTANCE:

The bacteria synthesize UDP-N-acetylmuramic acid pentapeptide and UDP-N-acetyl glucosamine. The peptidoglycan residues are linked together and form a long strand and UDP is split off, finally the cross linking of peptidoglycan residues between neighbouring strands is achieved by cleavage of terminal D-alanine of peptide chains by transpeptidases and cell wall rigidity is maintained[2].

The main drugs used in treatment of staphylococcus aureus skin infection are beta-lactam antibiotics (penicillins, cephalosporins, monobactams and carbapenems)[35]. Staphylococcus aureus are resistant to many antibiotics, these include beta-lactam antibiotics, glycopeptide antibiotics (Vancomycin), ribosomal inhibitor like macrolide lincosamide, streptograminB (erythromycin,clindamycin), aminoglycosides (gentamicin, amikacin), tetracyclines (doxycyline), DNA gyrase blocking drugs (quinolones) and antimetabolite trimethoprim sulfamethoxazole[11].

Beta-lactam antibiotics interfere with synthesis of bacterial cell wall by inhibiting transpeptidases so that cross linking in cell wall is inhibited[14]. These enzymes and related proteins are called as penicillin binding proteins (PBPs) located in bacterial cell membrane[11]. Penicillin was the first

antibiotic to be used clinically in 1941 and it was the drug of choice for treatment of serious staphylococcus aureus infections. Penicillinase a narrow spectrum beta lactamase emerged, due to acquisition of plasmid borne genetic elements coding for beta lactamases which opens beta lactam ring and inactivates penicillin[14]. More than 80% staphylococcus aureus isolates became resistant to penicillin[36].

Then penicillinase resistant penicillins (methicillin,cloxacillin) came into use by 1950 and were used only in staphylococcal infections. Later on extended spectrum penicillin was also produced, they were active against most gram positive and many gram negative organisms[35]. During 1980s resistance to penicillinase resistant penicillin emerged. The resistance appeared due to the presence of an altered penicillin binding protein called penicillin binding protein (PBP)[11].

There are about six different types of PBPs which are mostly transpeptidation enzymes[14]. This PBPs are under chromosomal control and mutation may alter their number and also their affinity for beta lactam drugs[14]. The PBPs resulted due to acquisition of a chromosomal gene called mecA[14]. Resistance to methicillin indicates resistance to all semisynthetic penicillins and also for many cephalosporins, erythromycin and clindamycin[11].

Methicillin resistant staphylococcus aureus gene contains a resistance island called staphylococcal chromosome cassette (SCC)mec[11]. Here mec is the genetic element that confers resistance to methicillin. Staphylococcal

chromosome cassette is an exogenous piece of DNA, which allows integration at homologous sites in the chromosome[14]. The critical genes in SCC mec are recombinases ccrA , ccrB and mecA gene[14]. This mecA encodes for a particular PBP called PBP2. The mecA gene may be expressed by some or all cells in a given population, the resistance mediated by altered PBPs is called heteroresistance[11]. Some staphylococcus aureus lack mecA gene but exhibit reistance to penicillinase resistant penicillins. This type of resistance is due to hyperproduction of beta lactamase enzyme which results in slow hydrolysis of semisynthetic penicillin[11].

Kacou- N douba et al (2011) study states PCR as the gold standard method to detect mec A gene in MRSA[37].

There are six types of SCCmec according to structure of their ccrA-ccrB and mecA complexes[14]. Types I, II and III belong to health care associated methicillin resistant staphylococcus aureus clones (HCA-MRSA), they harbour multiple resistance determinants and are large in size so they cannot be mobilized easily[11]. Types IV, V and VI are usually associated with community associated clones (CA-MRSA), they are smaller and do not carry multiple resistance genes and are more prone to mobilization[14].

Study by Shireen Furtado et al (2014) shows the increasing trend of CA-MRSA strains among pyoderma cases in India[25].

The CA-MRSA strains have other determinants in them which includes prophage – related Panton-valentine leucocidin(PVL) , multiple

staphylococcal exotoxin(set)genes and some strains have acquired arginine catabolic mobile element(ACME) inserted in downstream of the SCCmec cassette[38]. Panton valentine leucocidin along with γ-haemolysin forms a toxic component which has varying degree of haemolytic activity and lyse leukocytes efficiently[11]. This leukocidal activity is exerted directly on human polymorphonuclear cell membranes, causing degranulation of the cytoplasm, cell swelling and lysis. The toxin acts by causing the formation of pores, thereby altering cellular permeability to potassium and other cations[38].

After detailed study on CA-MRSA it is stated that they have emerged from HCA-MRSA but formed independently by acquiring SCCmec from coagulase negative staphylococcus aureus (CoNS)[38].

The prevalence of MRSA strains both HCA-MRSA and CA-MRSA has increased to great extent and geographical variation exists[39]. CA-MRSA is different from hospital strains in epidemiological and molecular aspect[38]. HCA-MRSA are isolated mainly in postoperative wound infections, catheterised patients and in hospital related pneumonia and bacteremia[38]. CA-MRSA strains are not associated with any risk factors, usually cause skin and soft tissue infections and rarely rapid fatal necrotizing pneumonia, necrotizing fasciitis, bone and joint infections[38].

CA-MRSA study done by Michael Z.David et al (2010) stated that children are at high risk of acquisition of infection by SCC mec type IV[38].

After emergence of MRSA strains, glycopeptide antibiotics became the drug of choice in treating these severe infections[40]. But by May 1996, Vancomycin intermediate staphylococcus aureus (VISA) was reported in Japan[14]. The first isolated strain labelled as Mu50, was recovered from a 4 month old child with sternal wound infection after cardiac surgery. Then subsequently these strains were isolated and reported from many countries[14]. First they were labelled as glycopeptide intermediate staphylococcus aureus (GISA) and later they were labelled as VISA[14].

Study by P Bhateja et al (2005) shows the prevalence of VRSA and its detection methods[41].

Vancomycin acts by inhibiting cell synthesis by binding to D-ala-D-ala terminal and block both transpeptidation and transglycosylation[14]. The VISA strains arises from chromosomal mutations that affect the structure of the cell wall peptidoglycan[14]. VISA strains have thick cell wall that contains increased number of free uncrosslinked D-ala-D-ala terminals and these act as traps before glycopeptide molecules reach their target[14]. The CLSI (Central laboratories standards institute) defines staphylococcus aureus requiring Vancomycin concentration of $\leq$ 2µg/ml for growth inhibition as susceptible, those requiring 4 to 8µg/ml as intermediate and those requiring $\geq$16µg/ml as resistant[42].

CA- MRSA strains pose a major threat to the community[44]. So local disinfection in carriers is done with application of mupirocin2% ointment[45,47]. Thus inappropriate use of this antibiotic has led to the

emergence of mupirocin resistant strains such as Low level resistance (MIC 8 to 256 mg/l) and high level resistance (MIC≥512mg/l)[14,49].

SK Oommen et al (2010) study on Mupirocin resistance in Staphylococcus spp shows emergence of High level Mupirocin resistance among MRSA isolates[47].

Many staphylococcus aureus have developed reistance against protein synthesis inhibitors mainly macrolide-lincosamide-streptogramins(MLSb)[48]. These antibiotic molecules bind to bacterial ribosomes and block protein synthesis. Ribosome modification and drug efflux are the mechanism responsible for development of resistance in staphylococcus aureus[14]. Ribosome modification is mediated by erm gene which codes for erythromycin methylase that decreases the affinity of the drug to its target[44]. These erm determinants are located in mobile genetic elements such as transposons or plasmids. In staphylococcus aureus the expression of erm gene is inducible[44]. The erm product is synthesised only in the presence of inducing drugs. Among MLSb drugs (erythromycin, clindamycin and streptogramins) macrolides are good erm inducers[14]. Once if they are induced the gene product confers cross resistance to other members of MLSb group of drugs, this type is called inducible resistance (i.e,diffusion of erythromycin towards clindamycin disk induces clindamycin resistance as a result the zone of inhibition around clindamycin takes D-shape)[44]. But in some strains the erm gene undergoes mutation and results in constitutive resistance expression (i.e,no inhibition zone around clindamycin)[44].

STREPTOCOCCUS PYOGENES:

The pathogenic streptococcus pyogenes (group A streptococcus) is one of the most important bacterial pathogens of humans[61]. This ubiquitous organism is associated with two non-suppurative sequelae, acute rheumatic fever (ARF) and post streptococcal acute glomerulonephritis (AGN)[63].

Study by Axana Haggar et al (2012) shows high number of streptococcal pharyngitis in north India whereas streptococcal pyoderma was frequently recorded in south India[62].

In 1874 Billroth was the first person to demonstrate streptococci from case of erysipelas and wound infection[14]. Later Pasteur in1879 isolated streptococci from the blood of a patient with puerperal sepsis. And in 1884 Rosenbach named this organism as streptococcus pyogenes[14].

The classification of streptococci is based on 1) colony morphology and haemolytic reactions on blood agar, 2) serologic specificity of the cell wall group specific substrate (Lancefield antigens) and other cell wall or capsular antigens, 3)biochemical reactions, 4) ecologic features[31]. The group specific substance (carbohydrate) is present in the cell wall of many streptococci and forms the basis of serological grouping into Lancifield groups A-H and K-U. The serological specificity of carbohydrate is due to the presence of specific amino sugar[30].

In streptococcus pyogenes the specific amino sugar is rhamnose-N-acetylglucosamine,for group B it is rhamnose-glucosamine polysaccharide and for group C it is rhamnose-N-acetylgalactosamine[14]. Typing is done

only for groups A, B, C, F and G because they are responsible for disease in humans and extracts of group specific antigen available for typing[11].

Morphology and general features:

Streptococcus pyogenes are spherical or ovoid cells 0.6 to 1.0 µm in diameter and are arranged in pairs and chains[30]. The length of the chains vary widely and are determined by environmental factors, long chains are formed when they grow in serum or blood[11]. They are gram positive, non- motile, non- spore forming, catalase negative, and facultative anaerobic[14]. They are fastidious organisms and grow in media which contain blood or serum[31]. They are insoluble in bile and ferments lactose and mannitol and PYRase positive[14]. Many group A strains produce capsules composed of hyaluronic acid and appear mucoid and this protects the organism from phagocytosis[11].

The streptococcus pyogenes do not grow in simple media like nutrient agar, but grow on blood agar plates[11]. They appear as white to gray colonies 1 to 2mm in diameter surrounded by zone of complete hemolysis (β hemolysis)[11].Most strains grow well in 37 c. Streptococcus pyogenes exhibit either glossy or matte colonies. Strains in matte colonies produce more M protein and are virulent while strains in glossy colonies possess little M protein and are avirulent[11].

Streptococcus pyogenes cell wall contains protein antigens (M, T, R), group specific carbohydrate and peptidoglycans[11]. Hair like projection

through the capsule is made up of M protein and covered with lipoteichoic acid[11].

VIRULENCE FACTORS:

The group A streptococcus pyogenes possesses in it a number of cell surface components and extracellular products important in both the pathogenesis of infection and the human immune response[11]. Lipoteichoic acids acts as an important structure for promoting adherence of group A streptococci to pharyngeal epithelial cells. Other adhesive structures are fibronectin binding proteins F1(FBPs), protein F2(SbfII), FPB54 and PFBP these proteins promote adherence of streptococci to both pharyngeal and cutaneous cells[11].

M protein:

Serotyping of group A streptococci were carried on basis of M protein precipitin reactions (Lancefield) or T protein agglutination reactions (Griffith). M protein is a major somatic virulence factor present in streptococcus pyogenes[11]. M proteins are acid and heat stable, trypsin-labile, fibrillary proteins present in outer surface of the cell wall of streptococcus pyogenes[14]. M protein is a filamentous macromolecule and made of two polypeptide chains which are held together in alpha helical coiled configuration[63]. M protein is anchored in the cell membrane and extends through the peptidoglycan layer and projects from the surface of the bacterial cell. The amino acid sequence is specific among group A streptococcal strains[11].

The strains rich in M protein are resistant to phagocytosis and intracellular killing by polymorphonuclear leukocytes and multiply rapidly in fresh human blood, and initiate disease. The strains lacking M protein are avirulent and they are readily phagocytosed[67]. M protein acts by interfering with opsonisation of bacterial cells via inhibiting activation of the alternate complement pathway. There are two structural classes of M protein, classes I and II[67]. The M proteins form complexes with fibrinogen and bind to β2 integrins of neutrophils and release inflammatory mediators that induce vascular leakage, a major pathologic event seen in streptococcal toxic shock[11].

The M proteins play a role in adherence to keratinocytes in skin via interaction with the keratinocyte membrane cofactor CD46. Other surface antigenic substance are T, R protein[67].

Opacity factor:

Serum opacity factor (OF) is a protein antigen closely associated with M protein of group A streptococci. The OF is an α-lipoproteinase, it has the ability to opacify horse serum and possess fibronectin binding properties. OF is type specific[63].

Dwight R .Johnson et al (2005) study shows strain characterization of GAS by M protein, SOF and emm-gene sequence typing method[67].

Hemolysins:

The group A streptococci produce many extracellular products. Two distinct hemolysins have been elaborated[30]. Streptolysin O is oxygen labile, it is inhibited by oxygen reversibly and irreversibly inhibited by cholesterol[11]. It has toxic effects on erythrocytes and is toxic to many cells and cell fractions like polymorphonuclearleukocytes, platelets, tissue culture cells, lysosomes and to isolated mammalian and amphibian heart[11].

Streptolysin O is produced by most strains of streptococci and is antigenic[30]. So measurement of streptolysin O as ASO antibodies in human sera serves as an indicator of recent streptococcal infection. Streptolysin O produces hemolysis in subsurface colonies in pour plates[11].

Streptolysin S is the hemolysin produced by streptococcal strains that grow in the presence of serum, serum albumin, α-lipoprotein, ribonucleic acid and tween. Streptolysin S is nonantigenic and has many properties similar to streptolysin O, like damaging the membranes of polmorphonuclear leukocytes, platelets and sub cellular organelles[11]. It is oxygen stable and thermostable. Streptolysin S is responsible for producing hemolysis on the surface of blood agar plate[30].

Enzymes:

These enzymes serve as extracellular products that facilitate liquefaction of pus and spreading of streptococci through tissue planes which is seen in streptococcal cellulitis and necrotizing fasciitis[11]. They are (1) streptococcal deoxyribonucleases A, B, C and D, they degrade the

deoxyribonucleic acid and aid in spread of streptococci in tissues by liquefying pus[30]. Antibodies against DNase B(anti-DNase B) serves as a serological marker of prior group A streptococcal pharyngeal or skin lesions[30].(2) hyaluronidase depolymerizes hyaluronic acid found in the ground substance of connective tissue and this results in contiguous spread of the organism.(3)Streptokinase, promotes dissolution or hydrolysis of fibrin clots by catalysing the conversion of plasminogen to plasmin and may function by preventing the formation of fibrin barriers at the spreading edges of streptococcal lesions.(4) Pyrolidonyl peptidase(PYRase) ,these enzymes produced by streptococcus pyogenes hydrolyze L-pyrolidonyl-β-napthylamide and show positive reaction[1].

Toxins:

Pyrogenic exotoxins (SPE) are produced by many strains of streptococcus pyogenes. Two types of exotoxin are produced A and B, both are responsible for rash of scarlet fever. SPE B is C5a peptidase, this cleaves and inactivates C5a the chemotactic complement component which recruits polymorphonuclear cells to the site of infection[11]. This SPE carries a lysogenic phage that has been associated with streptococcal toxic shock syndrome and scarlet fever. These pyrogenic exotoxins act as super antigens by stimulating T cells and binding to class II MHC[11].

Skin infections caused by streptococcus pyogenes:s

The β-haemolytic streptococci cause many skin infections. But important among them are impetigo, blistering distal dactylitis, ecthyma, erysipelas, necrotizing fasciitis ad septicaemia[3].

Clinical significance:

Streptococcus pyogenes are seen only in humans, because they are the natural reservoir and the organism is transmitted from one person to the other by respiratory route and skin inoculation[11].

Acute glomerulonephritis is an inflammatory disease of renal glomerulus that is associated with diffuse glomerular lesions, hypertension, haematuria and proteinuria[14]. Glomerulonephritis occurs as early as 10 days following pharyngitis, sometimes 3 to 6 weeks following skin infections. Laboratory findings include decreased haemoglobin, elevated erythrocyte sedimentation rate, decreased C3 and total complement, haematuria and proteinuria[50]. Streptococcus pyogenes M types M1,M3,M5, M16,M18,M19 and M24 have proved to be rheumatogenic. Strains belonging to certain M types M2,M49,M55,M57,M59,M60 and M61 have been associated with glomerulonephritis following skin infections[72].

M,Palani Kumar et al (2004) study shows Biotype 10 of group A Streptococci being isolated from children with pyoderma[64].

Treatment:

Most streptococcus pyogenes respond to penicillin G and most are sensitive to erythromycin, dicloxacillin. Patients who are allergic to

penicillin are treated with erythromycin and cephalexin[28]. Topical mupirocin ointment is also effective. Now strains resistant to erythromycin have emerged. They are resistant to sulphonamides. In severe infections linezolid and Vancomycin are the drugs of choice[28].

ENTEROCOCCI:

The genus enterococcus was previously classified under group D streptococci[30]. More than 90% of enterococcus strains possess Lancefield group D lipoteichoic antigens in their cell wall[11]. Enterococcus are present as normal resident flora of gastrointestinal and biliary tracts and in less numbers in vagina and male urethra[14]. They are seen as the second most common isolates of nosocomial urinary tract and wound infection and most common cause of nosocomial bacteremia[11].

Paudel U et al (2013) study shows involvement of Enterococcus faecalis in pyodermal lesions[6].

There are about 37 species of enterococci, very few species affect human beings[11]. Enterococcus faecalis is the common isolate which accounts for 80-90% followed by Enterococcus faecium 10-15% from human enterococcal infections[14]. Other enterococcal species are rarely encountered in human specimens[14].

Cultural characters:

They are gram positive cocci larger than Streptococcus pyogenes, facultative anaerobic and appear oval in shape[11]. Usually arranged as single cells, pairs and short chains, non-motile and non-capsulate. Enterococci are differentiated from other streptococcus species by their ability to grow in ordinary nutrient agar and Mac Conkey agar[31]. Grows as small translucent colony in nutrient agar and as small magenta coloured colonies in MacConkey agar, as alpha haemolytic or non-haemolytic colonies in blood agar[11].

Enterococcus has the ability to survive heating at 60 degrees for 30 minutes and grow within a wide range of temperatures (10-45° C)[31]. They grow on media with high salt content 6.5% NaCl and can grow at pH 9.6 and has the ability to hydrolyze esculin in presence of 40% bile[31]. It also produces pyrrolidonyl arylamidase(PYR)[31].

Biochemical characters:

Enterococcus faecalis ferments and produces acid from glucose, mannitol, sorbitol, sucrose and pyruvate. Also possess arginine dihydrolase and produces ammonia from arginine[31].

Virulence Factors:

Aggregation substance (AS) is a surface –bound, plasmid encoded protein which promotes clumping of organisms and facilitates plasmid exchange[11]. Some strains produce Esp(ectracellular surface protein) which helps the organism to evade immune activity. Most strains produce

extracellular superoxide that enhances the virulence in mixed-flora abscesses[11]. Coccolysin acts by inactivating endothelin, a vasoactive peptide[11]. The cytolysin is a hemolysin which is capable of lysing eukaryotic and prokaryotic cells[11].

Risk Factors for acquisition of Enterococcal infection:

They are immunosuppression, advanced age, underlying disease (e.g., prematurity, diabetes, malignancy, congestive cardiac failure, renal insufficiency, deep seated infections) and gastrointestinal, genitourinary or respiratory tract instrumentation and long term hospitalization[11].

MATERIALS & METHODS

MATERIALS AND METHODS

TYPE OF STUDY:

A hospital based prospective cross-sectional study.

PLACE OF STUDY:

The study was conducted in outpatient department of Dermatology in collaboration with the Department of Microbiology, Coimbatore Medical College and Hospital, Coimbatore.

DURATION OF STUDY:

The study was conducted over a period of one year, from August 2014 to July 2015.

AGE GROUP:

The patients for our study were in paediatric age group (0-12 years).

APPROVAL:

Ethical clearance was obtained from Institutional Ethics Committee and informed consent was obtained from parents or guardian accompanying the children for treatment.

SAMPLE SIZE:

The sample size of my study is 200 cases of Pyoderma in Paediatric age group attending Dermatology OPD.

STUDY ELIGIBLITY CRITERIA:

Inclusion Criteria:

1) Children below 12 years with purulent skin lesions diagnosed as either Primary or Secondary Pyoderma by the Dermatologist.
2) Children of both gender without hospitalization in the past one year and those not on antibiotic treatment in the previous two months.

Exclusion Criteria:

1) Children above 12 years.
2) Children who had received prior topical or systemic treatment for the presenting skin lesions.
3) Patients of resolving Pyoderma.

EVALUATION:

A detailed history from the parent regarding Information about economic status of the family, income of parent, nutritional status, duration of presenting illness and history of similar skin problems in the past or any history of similar illness within the family members was collected. Examination of the lesion including morphology of the lesions, their anatomical distribution and associated discharge, crusting or scaling was done.

Specimen Collection and Transportation:

Children under 12 years with purulent skin lesion and those diagnosed as a case of Pyoderma either primary or secondary by Dermatologist on basis of clinical grounds were selected as study subjects. The parent were explained about the microbiological investigation of the lesion and informed consent obtained.

In **Severe crusted lesions** the site was cleaned with 70% alcohol solution. The crust removed or lifted using sterile cotton and forceps and the area underneath was touched with two sterile cotton swabs. And the swabs were placed inside a sterile plastic swab container.

In **open ulcers and discharging wounds** the debris was cleaned using normal saline. The area surrounding the lesion was cleaned with 70% alcohol without touching the wound, to prevent contamination by skin commensals. The swabs were moistened with normal saline before collection of specimen to increase the isolation of organisms from the site. Then two sterile swabs were rubbed over the advancing edge of the ulcer with pus or discharge from the site. The swabs were placed in a sterile container.

In **intact pustules**, first the site and surrounding area were cleaned with 70% alcohol. The cleaning activity was performed starting from centre (over intact pustules) and then advanced to surrounding area in a circular manner from centre to periphery, so that contamination by skin commensal

is prevented. Then by using a sterile needle the intact pustule was ruptured. And the expressed pus touched with two sterile cotton swabs.

The specimen collected by sterile aseptic precautions was placed in a sterile tube, properly labelled with name, age, gender, OP number and date. Then the swabs were transported to the Microbiology laboratory immediately.

SPECIMEN PROCESSING:

Gram's Stain:

One swab was exclusively used for preparing direct smear immediately after collection of specimen. The sterile glass slide is cleaned, exposed to bunsen flame and then allowed to cool. And then a direct smear of size 2*1 cm is made over the slide[31]. The side of the smear and sample number is marked with a glass marking pencil. The smear is allowed to air dry. Then the smear is heat fixed by passing the under surface of the slide gently over the flame[31].

In the heat fixed slide Gram staining was done. First methyl violet (primary stain) was poured over the smear and allowed to stand for one minute[1]. Then washed and mordant Gram's Iodine is allowed to stand for one minute. Then washed and decolouriser Acetone added in drops under thin stream of running tap water[1]. After that dilute carbol fuchsin is flooded over the smear and allowed to stand for one minute. Finally the slide is washed, the smear is air dried and viewed under oil immersion method in 100x objective[1].

CULTURE ISOLATION:

The second swab was used for culture of pathogens. The swab was streaked in Nutrient agar, Blood agar and MacConkey agar. The plates were incubated aerobically at 35-37ºC for 24 hours.

The organism were identified by their colony morphology, Gram staining and following biochemical reactions.

- Catalase.
- Modified Oxidase.
- Methyl red and Voges-Proskauer test.
- Indole test.
- Bacitracin susceptibility test.
- Urease test.
- Slide and Tube coagulase test.
- Bile esculin agar.
- Growth at 10 and 45ºC for Enterococci.
- PYR test.
- Sugar fermentation test.

1) CATALASE TEST:

The colony to be tested was transferred by a sterile wooden stick to the glass test tube containing 30% hydrogen peroxide. Appearance of brisk effervescence by liberation of nascent oxygen immediately was taken as positive test. This test was performed with Staphylococcus aureus

(ATCC25923) as positive control and Streptococcus as negative control. S.aureus catalase positive and Streptococcus pyogenes, Enterococcus faecalis were catalase negative[1].

2) METHYL RED TEST(MR):

This test is used in identification of organism which produces and maintain stable acid end products from glucose fermentation and, by overcoming the buffering capacity of the medium.

The colonies to be tested were inoculated into MR/VP broth and incubated at 35 degree for 48 to 72 hours. Then 5drops of MR reagent was added to the broth. Appearance of red colour at the surface of the medium is considered positive. Staphylococcus aureus is MR positive[11].

3) VOGES PROSKAUER TEST(VP):

VP test is used in identifying the organisms which produces neutral end product (acetoin) from glucose fermentation.

The isolate to be identified are inoculated into MR-VP broth and incubated at 35-37°c for 48 hours. First reagent A 0.6ml (alpha napthol 5%, absolute ethyl alcohol) is added, followed by 0.2ml of reagent B (40% potassium hydroxide). The tube is shaken gently without plugging, so that the medium is exposed to atmospheric oxygen. The tubes were allowed to stand undisturbed for 10-15 minutes. Pinkish red colour at the

surface of the medium is considered positive. Staphylococcus aureus is positive for both MR and VP tests[11].

4) COAGULASE TEST:

Staphylococcus aureus produces two types of coagulase Free and Bound coagulase. This Coagulase test serves as an important test to differentiate Staphylococcus aureus from CoNS. This enzyme has prothrombin like activity and converts soluble fibrinogen to insoluble fibrin. They are identified by slide and tube coagulase tests[31].

Slide coagulase:

Staphylococcus aureus produces bound coagulase or clumping factor which is attached to the bacterial cell wall[11]. First a drop of saline was placed on the slide. The colony to be tested is taken and a milky suspension was made by thoroughly emulsifying the colony in normal saline. The fresh plasma separated from EDTA blood, was added to the milky suspension using straight wire[11]. Clumps formed within 5-20 seconds is taken as positive. Staphylococcus aureus (ATCC 25923) is used as positive control and Staphylococcus epidermidis used as negative control[11].

Tube Coagulase:

Staphylococcus aureus forms free coagulase which is secreted in culture filtrates, binds to prothrombin (coagulase reacting factor) in plasma and becomes enzymatically active. This in turn reacts with fibrinogen to form insoluble fibrin clot.

First 0.5ml of plasma was taken in a sterile test tube and to that a 0.5 ml of overnight broth culture of the organism added and incubated at 37 degree for 4 hours. It is examined every 30 minutes for clot formation. Staphylococcus aureus (ATCC 25923) is used as positive control and Staphylococcus epidermidis used as negative control and plasma controls were also included[11].

5) BACITRACIN SUSCEPTIBILITY:

This test was done to identify and differentiate Streptococcus pyogenes from other β-haemolytic Streptococcus species.

The isolate to be tested was streaked in blood agar by sterile loop and bacitracin 0.04 units placed at the centre[1]. The plate was incubated at 35-37ºC in 5%-10% carbon dioxide atmosphere for 18-24 hours. A zone of inhibition more than 10mm is taken as positive (susceptible)[1].

6) BILE ESCULIN TEST:

This test was done to differentiate group D Streptococcus and Enteroccocus from other Streptococcal species. The alpha haemolytic colonies from Blood agar plate were inoculated onto the slant surface of bile esculin agar and incubated at 35-37 degree for 24-48 hours. The Enterococci and some group D Streptococci grow in presence of 4% bile and hydrolyses the esculin to esculetin. The esculetin reacts with ferric ammonium citrate in the medium and forms dark brown to black precipitate[1].

7) PYR TEST:

Streptococcus pyogenes and Enterococci isolates were subjected to PYR test. The bacterial colony inoculated into L- pyrrolidonyl-β-Napthylamide agar and incubated at 35-37 degree for 18-24 hours. Presence of the enzyme L-pyrrolidonyl arylamidase in Streptococcus pyogenes or Enterococcus faecalis hydrolyses the substrate and forms β-Napthylamine[31].

The colonies are picked up with a sterile cotton swab and a drop of PYR reagent (N-N-dimethyl cinnamaldehyde) was added to the swab. Development of red colour (positive reaction) within 5-10 minutes was observed[31].

8) SUGAR FERMENTATION TESTS:

It is done to test the ability of an organism to ferment a particular carbohydrate present in the medium with bromothymolblue as indicator. The test organism was inoculated and incubated for 16-18 hours. Fermentation is indicated by change in colour from blue to yellow[11].

ANTIBIOTIC SENSITIVITY:

The colonies confirmed as Staphylococcus aureus, Streptococcus pyogenes and Enterococcus faecalis by Gram stain and other biochemical reactions were selected and Antibiotic sensitivity testing (AST) performed.

Around three to five colonies of same morphological type were selected for antibiogram. They were inoculated in 4-5ml of nutrient broth. And incubated at 35-37 degree until the inoculum matches 0.5 McFarland standard[1].

For antimicrobial sensitivity testing of Staphylococcus aureus and Enterococci, Muller Hinton agar was used and for fastidious organisms like Streptococcus pyogenes MHA with 5% sheep blood agar, used for antimicrobial testing by Kirby- Bauer Disc Diffusion Method. In MHA pH maintained at 7.2 to 7.4, and the depth of the MHA agar maintained at 4mm[1].

The sterile swab soaked in the inoculated nutrient broth, was pressed in the side walls of test tube to remove excess inoculum and it was streaked over the entire agar surface. The same procedure was repeated three times by turning the plate at 60° angle between each streaking. So that even distribution is ensured. By using sterile forceps Antibiotic discs are placed in MHA plate as per CLSI guidelines[11].

The Antibiotic discs (HIMEDIA) was used and selected according to CLSI guidelines. Only five discs are placed in 90mm plate. The disc were placed over the agar surface by sterile forceps. A distance of 24mm from centre of one disc to other disc was maintained[11]. The discs were gently pressed to ensure complete contact with agar. Then the AST plates were inverted and kept in incubator within 15 minutes of placing disc. The plates were incubated at 35-37 degree for 16-18 hours[11].

Antibiotic disc used for Staphylococcus aureus were Cotrimoxazole (25µg), Penicillin10 units, Gentamycin(10µg), Amoxicillin(30µg), Erythromycin(15µg), Azithromycin(15µg), Clindamycin(2µg), Cephalexin(30µg), Cefoxitin(30µg), Doxycycline(30µg), Ciprofloxacin(5µg),

Linezolid(15µg), Teicoplanin(30µg) and Mupirocin(200µg). The zone size was measured and recorded by viewing the plate in reflected light[42].

For Streptococcuc pyogenes the following antibiotic discs were used, Bacitracin(0.04units), Cotrimoxazole(25µg), Penicillin10units, Erythromycin(15µg), Clindamycin(2µg), Doxycycline(30µg), Linezolid(15µg), Vancomycin(30µg), Ciprofloxacin(5µg), Ceftriaxone(30µg), Teicoplanin(30µg)[42].

The antibiotic discs used for Enterococcus faecalis were Ampicillin (10µg), Erythromycin (15µg), Ciprofloxacin (5µg), Doxycycline (30µg), Linezolid (15µg), Vancomycin (30µg), Teicoplanin (30µg),High level Gentamycin (120µg)[42].

PHENOTYPIC SCREENING TEST FOR STAPHYLOCOCCUS AUREUS:

The Staphylococcus aureus isolate was inoculated in nutrient broth and incubated till the inoculum reaches 0.5 McFarland turbidity, and following Phenotypic screening tests for were perfomed[14].

1) Screening test for **mec-A mediated Oxacillin resistance**:

A lawn culture was done in MHA plate as for standard disc diffusion by Kirby Bauer method[11]. Cefoxitin 30µg disc placed and incubated at 33-35ºC in ambient air for 16-18 hours. The AST plate was examined in reflected light, a zone of inhibition below 21mm is taken as mec-A positive (MRSA) and zone diameter more than 22mm is taken as mec-A negative (MSSA) strain[42].

Methicillin resistance is mediated by acquired penicillin binding protein 2A (PBP2A), encoded by chromosomal gene called mec-A[11]. PBP2A has special requirement for peculiar cell wall precursors.The strains positive by this test were labelled as Methicillin Resistant Staphylococcus aureus (MRSA). Cefoxitin is used as a surrogate marker for MRSA[42].

2) Inducible Clindamycin resistance D-test:

A lawn culture of Staphylococcus aureus isolate was done in MHA plate. Macrolide antibiotic Erythromycin 15µg disc was placed and Lincosamide antibiotic Clindamycin 2 µg disc placed 15-25 mm apart. The plate was incubated in 35-37ºC in ambient air for 16-18 hours[11].

Flattening of zone of inhibition around clindamycin adjacent to the erythromycin disc resulting in D-shape was considered positive[44]. The Inducible resistance is due to erm B gene of staphylococcus aureus and macrolides are potent erm B inducers[14]. If there is no zone of inhibition around clindamycin it is taken as Constitutive resistance[44].

3) High Level Mupirocin Resistance:

The Staphylococcus aureus isolate was streaked in MHA plate. Mupirocin 200µg disc placed in centre of the plate and incubated at 35-37ºC for 24 hours. The zone of inhibition was measured in transmitted light[42].

According to CLSI guidelines absence of zone of inhibition indicates High level Mupirocin resistance and presence of zone of any size is taken as absence of high level mupirocin resistance[42]. EUCAST (European

Committee on Antimicrobial susceptibility) has clinical break points as follows, zone diameter more than 30mm is taken as susceptible and zone diameter less than 18mm is taken as resistant.

4)Screening of **Vancomycin Minimal Inhibitory Concentration(MIC) by E test:**

Vancomycin disc diffusion does not differentiate between Vancomycin susceptible isolates from Vancomycin intermediate isolates[41]. The high molecular weight antibiotics like Vancomycin do not diffuse according to concentration gradient, while diffusing through the MHA medium[11].

MIC is the lowest concentration of antimicrobial agent that inhibits visible growth. Commercial method of MIC determination is done by E test (HIMEDIA Ezy MIC strip Van 0.16 -256 mcg/ml)[43].

A lawn culture of the inoculum was made in MHA plate and E strip was brought to room temperature[43]. By using the adhesive applicator stick the E strip is placed in the centre of the plate and the applicator stick removed gently. E strip will be adsorbed and will firmly adhere to the agar surface immediately[43]. The MHA plate is then incubated at 35-37°c for 24 hours[43]. MIC is determined by examining the point of intersection of bacterial growth with graded concentration of Vancomycin in the E- strip which has MICs in the range of 0.016 mcg/ml to 256 mcg/ml[42].

The interpretive criteria for identifying Vancomycin sensitive, intermediate and resistant Staphylococcus aureus by E test method are as follows:

- Sensitive if MIC less than 2 mcg/ml (VSSA).
- MIC in between 4-8 mcg/ml are considered intermediate resistant (VISA).
- MIC above 16mcg/ml by E test is considered as Vancomycin resistant Staphylococcus aureus (VRSA)[42].

GENOTYPING OF CA-MRSA:

A Duplex-PCR was performed to screen the presence of mecA and lukS/F-PV (which encode the Panton Valentine Leucocidin S/F biocomponent proteins) genes[72].

For rapid DNA extraction, 2-5 colonies were suspended in 100µl of molecular-grade water (Qiagen,Germany) and heated in boiling water bath for 10 minutes[72]. After centrifugation at 10,000 rpm for 10 minutes, 1µl of the Supernatant (DNA template) was added to 20µl of the PCR reaction mixture (Invitrogen,USA). The reaction was carried out using the following steps:

1. An activation step at 94°C for 5 minutes.
2. Followed by 30 cycles of initial denaturation which is done at
    i) 94°C for 30 seconds.

ii) 50°C for 30 seconds and

iii) 72°C for 30 seconds, ending with a final step at 72°C for 7 minutes.

3. This was followed by a holding step at 4°C.

The PCR products were analysed by Gel Electrophoresis with 2 per cent agarose in TBE buffer with ethidium bromide (5µl/ml) and visualised by UV-transillumination. A 100 base pair (bp) DNA ladder (Invitrogen,USA) was used as a marker. Duplex-PCR yielded products with sizes of 433 and 310 bp which corresponded to lukS/F-PV and mec-A genes respectively[72].

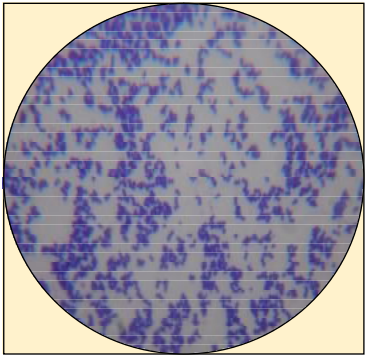

Figure 1: Gram Stain - Gram positive cocci in clusters of Staphylococcus aureus.

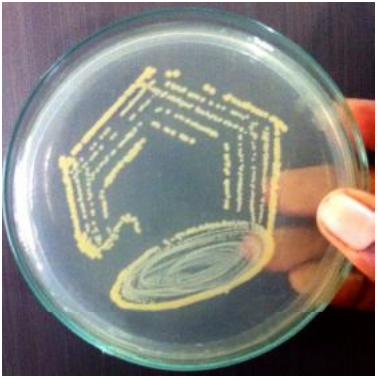

Figure 2: Nutrient Agar – Golden yellow colonies of Staphylococcus aureus.

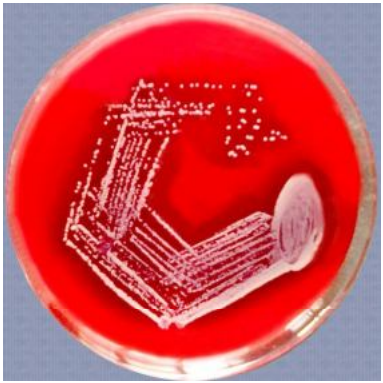

Figure 3: Beta hemolytic colonies of Staphylococcus aureus in 5% sheep blood agar.

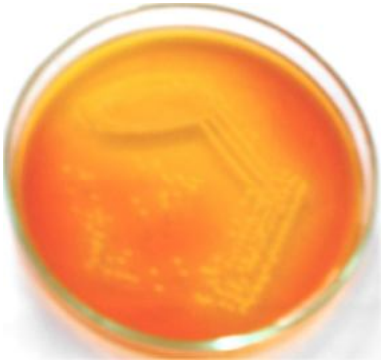

Figure 4: Mannitol Salt Agar– Yellow colonies of Staphylococcus aureus.

Figure 5: Antibiotic Sensitivity of Staphylococcus aureus by disc diffusion method

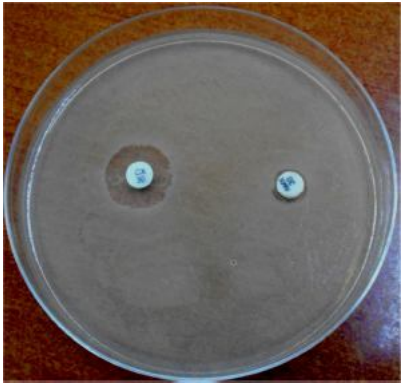

Figure 6: Phenotypic confirmation of MRSA using Cefoxitin disc

Figure 7: Inducible Clindamycin resistance D-Test

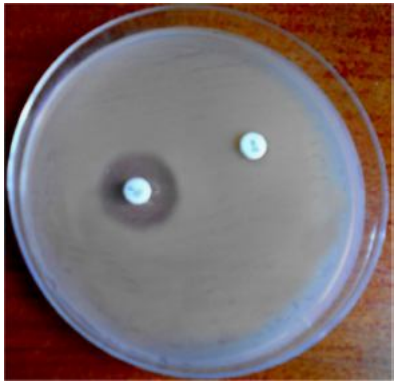

Figure 8: Constitutive Clindamycin resistance

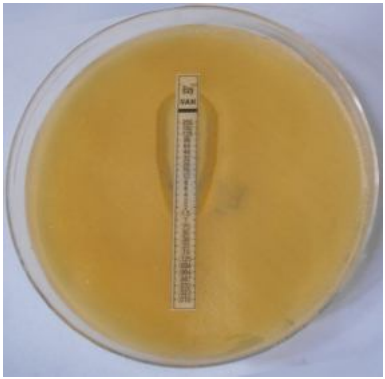

Figure 9: E Strip showing Vancomycin sensitivity of less than 2µg/ml for Staphylococcus aureus

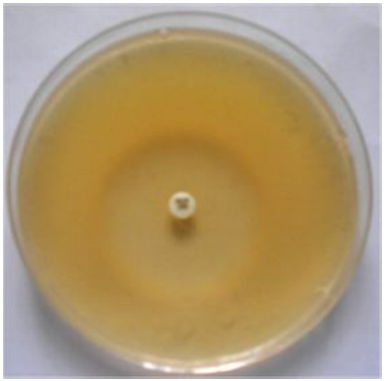

Figure 10: Mupirocin disc (200µg) Sensitive to Staphylococcus aureus

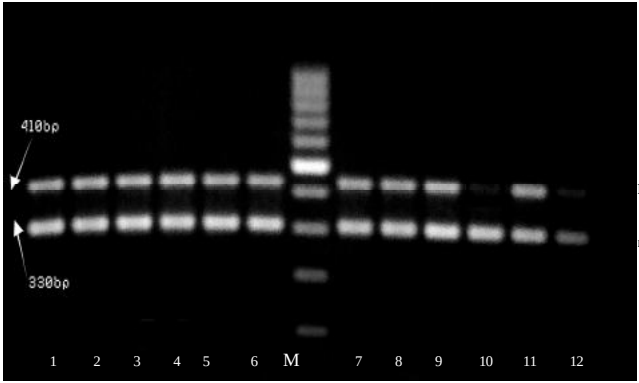

Figure 11: **Agarose gel electrophoresis of PCR products (mecA&pvl gene)**
Lane 'M' shows ladder molecular marker 100 bp
Lane 1 – 12 CA- MRSA Strains
Test sample showing 330 bp genes products for mecA gene and 410 bp for pvl gene

Figure 12: Child with Impetigo Lesion on Face

Figure 13: Beta hemolytic colonies of Streptococcus pyogenes in 5% Sheep Blood Agar

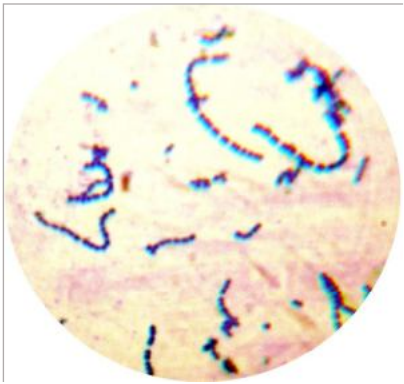

Figure 14: Gram positive cocci in chains - Streptococcus pyogenes

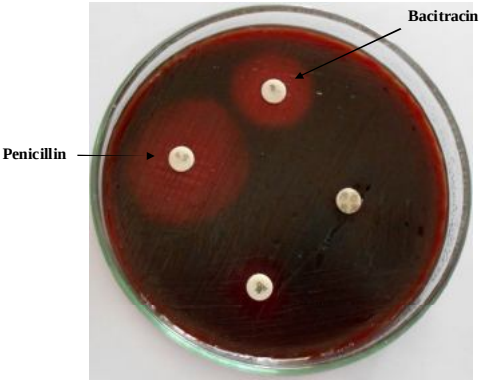

Figure 15: Streptococcus pyogenes – Bacitracin and Penicillin sensitivity

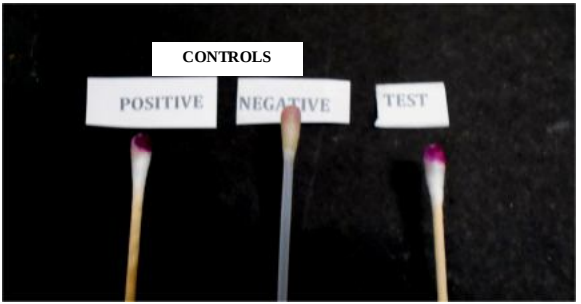

Figure 16: Streptococcus pyogenes - PYR Test

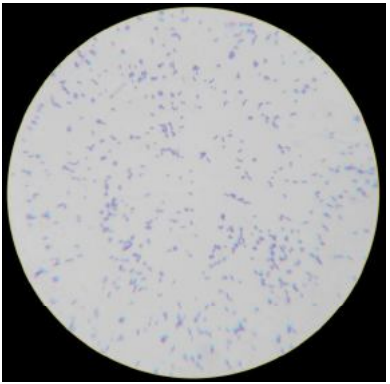

Figure 17: Gram Stain of Enterococcus faecalis

Figure 18: Bile Esculin Agar blackening

RESULTS

RESULTS

The study was conducted in **Dermatology outpatient** department in CMCH, Coimbatore. The study period was one year from July 2014 to June 2015. Two hundred children with signs and symptoms of Pyoderma were subjected to study. Both primary and secondary pyoderma were included.

The bacteriological trend and the antibiotic sensitivity pattern of the isolates done. The phenotypic screening tests for identifying MSSA and MRSA and reistance pattern of the isolates to Clindamycin, Mupirocin and Vancomycin was done

In our study, children less than 1month of age were 10.5% (n=21), 1month-1year 24.5% (n=49), 1year to 5year 44% (n=88) and 5-12 year group 21% (n=42). . Most of the children in preschool group were victims of Pyodermal lesions (table no.1).

Prevalence of pyoderma was more in male children 57% (n=114), than female children 43% (n=86) which constitutes a ratio of 1.3:1. Primary pyoderma cases were 72.5% (n=145) this was more common than Secondary pyoderma which constituted around 27.5% (n=55%).The incidence of primary pyoderma were observed high numbers in the pre-school (1-5years) age group. Secondary pyoderma were equally observed equally among pre-school and school going children (table no.1).

The pattern of **Primary pyoderma** observed in our centre shows **Impetigo** as the **single largest** group which accounts for 53.1% (n=77) cases. Followed by folliculitis with 20% (n=30%) and furunculosis and Ecthyma each 9.6%, Cellulitis 3.4% (n=5), Paronychia 2% (n=3) and Omphalitis 1% (n=2) given in table no.2.

Secondary pyoderma constituted 27.5% among the isolates. The common lesion observed in them were **Scabies** 45%(n=25) insect bite 34.5%(n=19), Contact dermatitis 10.9%(n=6) with secondary infection, Ulcer 3.6%(n=2) followed by one case 1.8%(n=1) each of Burns, pediculosis, intertrigo (table no.3)

Children attending Dermatology OP in our college were from in and around Coimbatore. Most of their parents or guardian were daily labourers, residing in urban slum area. Most of the pyodermal cases were from low income group 93.5% (n=187), lower middle income group 5.5% (n=11) and 1% (n=2) middle income group (Table no.6).

Pyodermal lesions were mostly observed in lower limb 43%, followed by uper limb 18.5%. Both upper and lower limb were involved in 12% of cases. Face in 8.5%, Ear lobule 5%, scalp and trunk each with 4.5%, buttock 2.5%. Umbilicus and axilla showed 1% involvement (table no.5).

In our study culture positivity was 91.5%(n=181) and no growth was seen in 9.5%(n=19) Among the culture positive cases Staphylococcus aureus was isolated as single largest group accounting for 76%(n=152).

Streptococcus pyogenes in 7% (n=14) Enterococcus faecalis in 1% (n=2) of cases. Mixed organism (Staphylococcus aureus + Streptococcus pyogenes) were observed in 6.5% (n=13) cases (table no.4).

Staphylococcus aureus was isolated as a single microorganism from most Primary and secondary pyodermal lesions (152 isolate). Streptococcus pyogenes was isolated from primary pyoderma lesions of Impetigo, Ecthyma, folliculitis and cellulitis (14 isolate). Two Enterococcus faecalis from case of cellulitis and scabies with secondary infection (table no.7).

Seasonal impact on pyodermal cases were studied which revealed maximum number of cases in the month of May 17%, April 15% followed by January 14% and June 12% cases in our area (table no.8).

Among the Staphlococcus aureus isolates penicillin was least sensitive1%, cotrimoxazole 48%, Gentamicin 63.1%,Amoxicillin 57.2%, Erythromycin 70%, Ciprofloxacin 72%, Clindamycin 80%, Cephalexin 78.9%, Azithromycin 82%, Cefoxitin 92%, Doxycycline 97% and Linezolid, Vancomycin, Teicoplanin, Mupirocin all showed 100% sensitive. Staphylococcus aureus from polymicrobial cases showed 100% cefoxitin sensitivity (table no.9).

Streptococcus pyogenes isolates were sensitive to Erythromycin 71.5%, Gentamycin 78.5%, Ciprofloxacin 85.7% and Bacitracin, Penicillin, Doxycyclline, Linezolid, Vancomycin, Ceftriaxone, Clindamycin all were 100% sensitive

Two Enterococcus faecalis isolates in our study. 50% of the isolates were sensitive to Ampicillin and Erythromycin. Ciprofloxacin, Doxycycline, Linezolid, Vancomycin, Teicoplanin and High level Gentamycin were 100% sensitive.

All the polymicrobial isolates encountered in our study were a combination of both S.aureus and St.pyogenes.

Mupirocin sensitivity among Staphylococcus aureus and CA-MRSA strains in our study showed all isolates were susceptible (100%) to this topical antibiotic (table.no.10).

Clindamycin resistance among the isolates in our study showed predominance of MSSA. Inducible clindamycin resistance was prevalent among MSSA15% (n=21) and 16.5% (n=2) among CA-MRSA strains. Constitutive clindamycin resistance 4% (n=6), were seen only in MSSA (Refer table no.11).

CA-MRSA isolated in our study was 7.8% (n=12).Most of the isolates were seen in folliculitis followed by scabies with secondary infection. CA-MRSA strains were least sensitive 33% to Cotrimoxazole and Gentamycin. Erythromycin 58%, 66.6% to Ciprofloxacin, Cephalexin and Azithromycin respectively. 83.3% to Clindamycin and 100% sensitive to Vancomycin,Linezolid,Teicoplanin,Doxycycline and Mupirocin. Gene typing was done by Duplex PCR method for 12 isolates and all were mec-A positive while 10 were positive for pvl gene (table no.12).

Statistical analysis was done to compare the antibiotic susceptibility pattern of Staphylococcus aureus and Streptococcus pyogenes isolates of primary pyoderma and secondary pyoderma. It was found to be statistically significant (table.no.13).

TABLE NO.1

AGE AND SEX WISE DISTRIBUTION

S.No	Age group	M	F	Total	Primary	Secondary
1	0 – 1 month	10	11	21	15	6
2	1 month – 1 year	28	21	49	39	10
3	1 – 5 year	56	32	88	67	21
4	5 – 12 year	20	22	42	24	18
TOTAL		**114**	**86**	**200**	**145**	**55**

TABLE NO.2

PATTERN OF PRIMARY PYODERMA

S.NO	DIAGNOSIS	NO.OF.CASES(n=145)	PERCENTAGE
1	IMPETIGO	77	53.1%
2	FOLLICULITIS	30	20%
3	FURUNCULOSIS	14	9.6%
4	ECTHYMA	14	9.6%
5	CELLULITIS	5	3.4%
6	PARONYCHIA	3	2%
7	OMPHALITIS	2	1.3%
TOTAL		**145**	**100%**

TABLE NO.3

PATTERN OF SECONDARY PYODERMA

S.NO	DIAGNOSIS	No.of.Cases (n=55)	Percentage
1	SCABIES WITH SECONDARY INFECTION	25	45%
2	INSECT BITE WITH SECONDARY INFECTION	19	34.5%
3	CONTACT DERMATITIS WITH SECONDARY INFECTION	6	10.9%
4	ULCER	2	3.6%
5	BURNS WITH INFECTION	1	1.8%
6	PEDICULOSIS WITH INFECTION	1	1.8%
7	INTERTRIGO WITH SECONDARY INFECTION	1	1.8%
TOTAL		**55**	**100%**

TABLE NO.4

COMPARSION BETWEEN CULTURE POSITIVE AND NO GROWTH

S.No	AGE	NAME OF THE ISOLATE				Sterile (No Growth)
		S. aureus	S. pyogenes	E. faecalis	Staph+ Strep	
1	0-1 month	15(9.8%)	3(21.4%)	-	1 (7.6%)	2(10.5%)
2	1 month-1 yr	36(23.5%)	4(28.5%)	-	4 (30.7%)	5(26.3%)
3	1 - 5 yrs	72(47.3%)	4(28.5%)	1(50%)	3 (23%)	8(42.10%)
4	5 - 12 yrs	29(19.07%)	3(21.4%)	1(50%)	5 (38.4%)	4(21.6%)
TOTAL		152	14	2	13	19

TABLE NO.5

SITE OF INVOLVEMENT OF PRIMARY AND SECONDARY PYODERMA

S.NO	SITE	No.of.Cases (n=200)	PERCENTAGE
1	LOWER LIMB	86	43%
2	UPPER LIMB	37	18.5%
3	BOTH(UL AND LL)	24	12%
4	FACE	17	8.5%
5	EAR LOBULE	10	5%
6	SCALP	9	4.5%
7	TRUNK	9	4.5%
8	BUTTOCK	5	2.5%
9	AXILLA	1	0.5%
10	UMBILICUS	2	1%
TOTAL		**200**	**100%**

TABLE NO.6

CATEGORISATION BY SOCIO ECONOMIC STATUS

S.No	Socio-Economic Status	No.of.Cases (n=200)	Percentage
1	LOW INCOME GROUP	187	93.5%
2	LOWER MIDDLE INCOME GROUP	11	5.5%
3	MIDDLE INCOME GROUP	2	1%
4	UPPER MIDDLE INCOME GROUP	-	-
5	HIGH INCOME GROUP	-	-

TABLE NO.7

CORRELATION BETWEEN CLINICAL TYPE OF PYODERMA

AND ORGANISM ISOLATED

Pyoderma	Clinical Type	S.aureus	S.pyogenes	Enterococci	STAPH+STREP
Primary Pyoderma	Impetigo	56	7	-	7
	Folliculitis	29	2	-	-
	Ecthyma	7	4	-	1
	Furunculosis	11	-	-	-
	Omphalitis	2	-	-	-
	Paronychia	3	-	-	-
	Cellulitis	3	1	1	-
Secondary Pyoderma	Scabies with sec infection	19	-	1	1
	Insect bite with sec infection	15	-	-	2
	Contact dermatitis with infection	4	-	-	1
	Ulcer with infection	1	-	-	1
	burns with infection	1	-	-	-
	Pediculosis with sec infection	1	-	-	-

TABLE NO.8

SEASONAL TREND

S.NO	MONTH	NO.OF .CASES	PERCENTAGE
1	SEPT 14	13	6.5%
2	OCT 14	7	3.5%
3	NOV 14	12	6%
4	DEC 14	12	6%
5	JAN 15	28	14%
6	FEB 15	20	10%
7	MAR 15	19	9.5%
8	APR 15	30	15%
9	MAY 15	34	17%
10	JUNE 15	25	12.5%
TOTAL		200	100%

TABLE NO.9

ANTIBIOTIC SUSCEPTIBILITY PATTERN OF ISOLATES

S.No	Name of the Antibiotic	S.aureus(n=152)		S.pyogenes(n=14)		E.faecalis(n=2)	
		S	%	S	%	S	%
1	Penicillin(10 units)	2	1	14	100%	-	-
2	Cotrimoxazole(25µg)	73	48%	0	0	-	-
3	Gentamicin(10µg)	96	63.1%	11	78.5%	-	-
4	Amoxicillin(30µg)	87	57.2%	-	-	-	-
5	Erythromycin(15µg)	107	70%	10	71.5%	1	50%
6	Ciprofloxacin(5µg)	109	72%	12	85.7%	2	100%
7	Clindamycin(2µg)	121	80%	14	100%	-	-
8	Doxycycline(30µg)	148	97%	14	100%	2	100%
9	Cephalexin(30µg)	120	78.9%	-	-	-	-
10	Cefoxitin(30µg)	140	92%	-	-	-	-
11	Azithromycin(15µg)	126	82%	-	-	-	-
12	Linezolid(30µg)	152	100%	14	100%	2	100%
13	Vancomycin(30µg)	152	100%	14	100%	2	100%
14	Teicoplanin(30µg)	152	100%	-	-	2	100%
15	Mupirocin(200µg)	152	100%	-	-	-	-
16	Bacitracin(0.04U)	-	-	14	100%	-	-
17	Ampicillin(10µg)	-	-	-	-	1	50%
18	Ceftriaxone(30µg)	-	-	14	100%	-	-
19	High level Gentamycin(120µg)	-	-	-	-	2	100%

TABLE NO.10

COMPARISON OF SUSCEPTIBILITY PATTERN OF MSSA AND CA-MRSA ISOLATES

S.NO	ANTIBIOTIC	MSSA(n=140)		CA-MRSA(n=12)	
		S	%	S	%
1	Penicillin(10 units)	2	1.4%	0	0
2	Amoxicillin(30µg)	87	57.2%	0	0
3	Cotrimoxazole(25µg)	69	49.2%	4	33%
4	Gentamicin(10µg)	92	64.7%	4	33%
5	Erythromycin(15µg)	100	71.4%	7	58.3%
6	Ciprofloxacin(5µg)	101	72%	8	66.6%
7	Clindamycin(2µg)	111	79%	10	83.3%
8	Doxycycline(30µg)	136	97%	12	100%
9	Cephalexin(30µg)	112	80%	8	66.6%
10	Cefoxitin(30µg)	140	100%	0	0
11	Azithromycin(15µg)	118	84%	8	66.6%
12	Linezolid(30µg)	140	100%	12	100%
13	Vancomycin(30µg)	140	100%	12	100%
14	Teicoplanin(30µg)	140	100%	12	100%
15	Mupirocin(200µg)	140	100%	12	100%

TABLE NO.11

CLINDAMYCIN RESISTANCE AMONG ISOLATES

TYPE OF RESISTANCE	MSSA (n=140)	CA-MRSA (n=12)	S. pyogenes
Inducible Clindamycin Resistance	21(15%)	2(16.5%)	--
Constitutive Clidamycin Resistance	6(4%)	-	

TABLE NO.12

CA-MRSA GENOTYPING

Lesion	**Percentage**	**Method**	**mec-A positive**	**pvl positive**
Folliculitis	58.3%	Twelve Isolates Subjected For Gene Study By Duplex Pcr Method	12(100%)	10(83.3%)
Scabies	25%			
Impetigo	8.3%			
Contact dermatitis with infection	8.3%			

TABLE NO 13

STATISTICAL ANALYSIS OF ANTIMICROBIAL SUSCEPTIBILITY BETWEEN ISOLATES OF PRIMARY AND SECONDARY PYODERMA

Organism	Antibiotic Susceptibility		p-Value*
	Primary (N=133) N%	Secondary (N=46) N%	
S.aureus	111 (83.46%)	41 (89.13%)	0.026
Strep.pyogenes	14 (10.53%)	-	
Both	8 (6.02%)	5 (10.87%)	

*__Fisher's exact test__ was used to assess the association.

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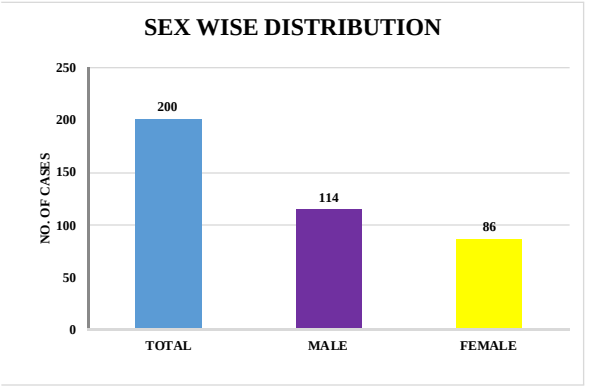

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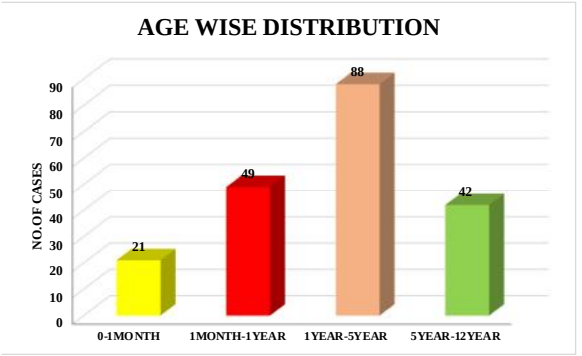

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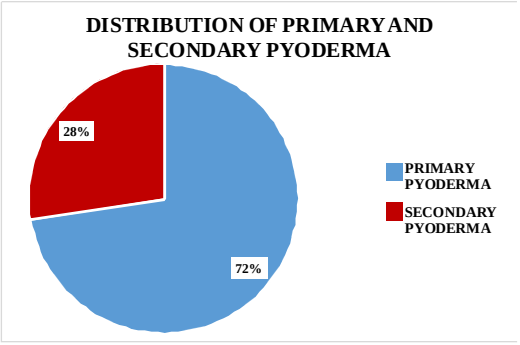

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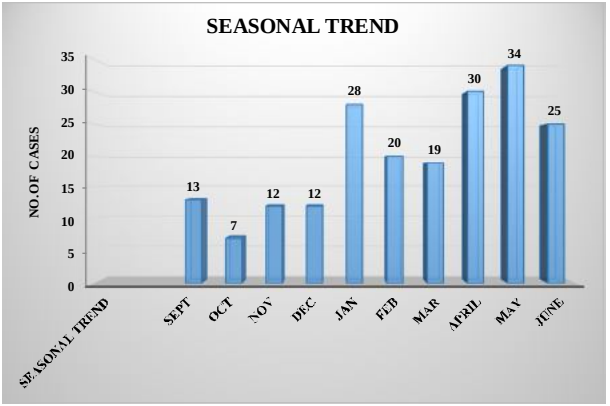

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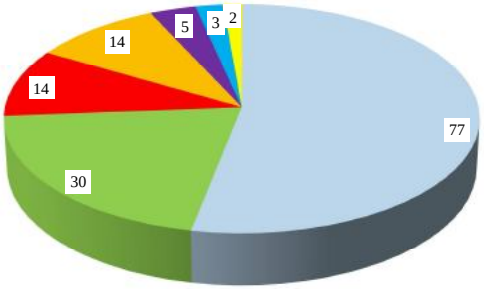
PATTERN OF PRIMARY PYODERMA

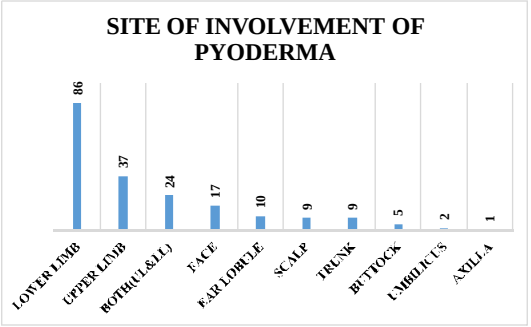


IMPETIGO **FOLLICULITIS** **FURUNCULOSIS**

ECTHYMA **CELLULITIS** **PARONYCHIA**

OMPHALITIS

CHART NO: 6



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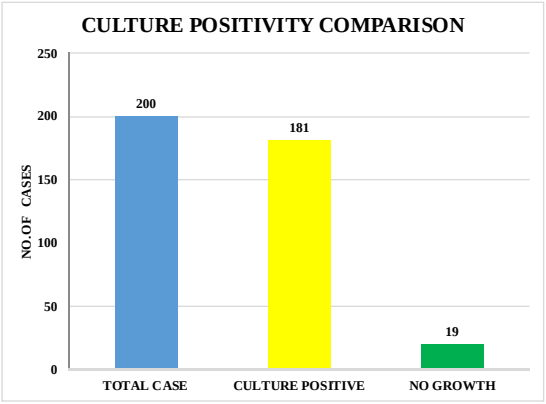

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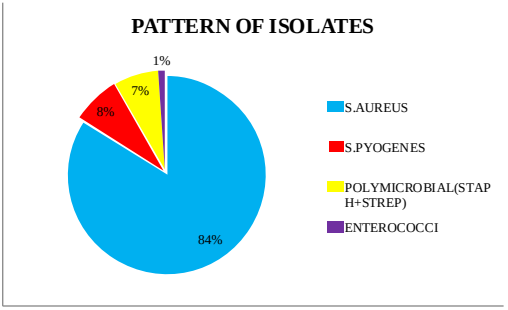

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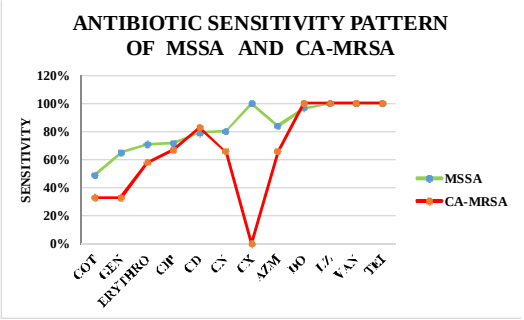

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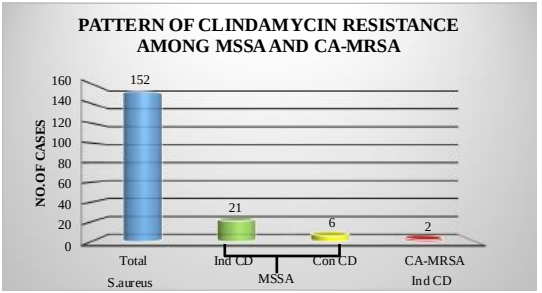
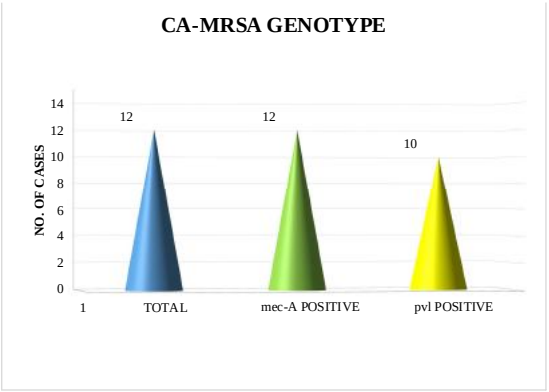


CHART NO: 11



DISCUSSION

DISCUSSION

Pyoderma is one of the common skin problems more prevalent among children[5]. The pattern of isolates and their susceptibility to antibiotics and resistance pattern are continuously changing[6,7]. So periodic survey of this problem in children living in a developing country like India becomes essential. This study was carried out at the Dermatology OPD among children less than 12 years suffering from pyodermal lesion.

Pyoderma cases were predominantly seen in male (57%) than female children (43%). This is in concordance with studies done by M Jyothi Nagmoti et al (1999), Rahul Patil et al (2006), Suresh K Malhotra et al (2012). Findings by Mathew et al,(1992) Kar et al, (1985) Ramani and Jayakar et al,(1980) who have shown a female predominance in Pyodermal cases are contradictory.The male predominance over female children as the incidental finding may be due to random selection of OPD cases and also due to regional variation.

Age predilection in our study shows a maximum number of cases in pre-school age i.e 1-5 years(44%) followed by school going age i.e 6-12 yr (21%). Our study correlates well with that of Kaliaperumal Karthikeyan et al (2004), Naresh Jain et al (2010) and Neirita Hazarika et al (2012).

Involvement of preschool children as seen in my study might be due to minor trauma caused mainly by insect bite. In most of these cases the parents were found to be labourers, living in slums where

environmental sanitation is poor. Parental care is also negligible and children seem to be more prone for infections.

The prevalence of Primary pyoderma was 72.5% while secondary pyoderma constituted 27.5% of cases in our study. This correlates well with that of RG Baslas et al (1990), Kakar et al (1999), Shireen Furtado et al (2012), Dr Sanjiv V Choudhray et al (2013), Janardhan B et al (2015). But Suresh K Malhothra et al (2014) study showed a predominance of secondary pyoderma in his studies.

Impetigo was the predominant skin lesion among all pyoderma. Prevalence of malnutrition, insect bite and lack of awareness are the factors contributing to this. In our region, too hot a summer prevails for a longer period which also predisposes for bacterial infection.

Our study shows Scabies with secondary infection (45%) as a common lesion in secondary pyoderma followed by insect bite 34.5%.These findings corresponds to findings of Dr Baijayanti Baur et al (2013), Naresh Jain et al (2010), Baskaran et al (1989), RG Baslas et al (1990) whereas a study by Janardhanan B et al (2015) have ranked Eczema with infection as the commonest.

Most of our patients were from low socioeconomic strata where overcrowding and poor personal hygiene are common factors predisposing to scabies as a common skin lesion. Moreover sharing of towels,blankets and soaps between various family members provides an easy chance for spread of scabies infection.

Another factor could be the intense itching associated with burrowing of Sarcoptes scabiei an itch mite which predisposes to secondary bacterial infection in these lesions.

Our study shows lower limb (43%) as the major site for pyodermal lesions which is in accordance with the findings of Kacou N douba et al (2011), Shashi Gandhi et al (2012) and differs from that of studies by Neirita Hazarika et al(2012), Kharel C et al (2005).Lower limb involvement in more cases is due to scanty clothing, climatic condition and another reason is that the Children living under poor sanitary conditions are mostly exposed to mosquito and insect bites of the exposed parts.

The seasonal variation pattern in our study shows maximum number of cases enrolled in summer. May and April were highest with 17% and 15%. Our observation correlates well with findings of Suresh K Malhothra et al[10] (2012), Hermann Feldmeier et al[12] (2005), S Mariette Mathew et al[36] (1992). The reason for high prevalence in summer might be because of sweating. The children are tempted to scratch their skin very often and traumatize them; bacteria invade the damaged skin and colonize easily.

The pattern of isolates shows Staphylococcus aureus (76%) to be the major isolate, Streptococcus pyogenes 7%, Enterococcus faecalis 1%, mixed growth 6.5% and 9.5% of cases were culture negative. Similar patterns were shown by Adarsha Chopra et al (1994), Shashi Gandhi et al[7] (2012), Neirita Hazarika et al[5] (2012). The study by Varsha T Kalsheti et al (2014) and Rahul Patil et al[60] (2006) shows S.aureus as the

predominant isolate among pyoderma while Streptococcus pyogenes was predominant isolate in studies by Mallik et al[24] (2006) and Shelby-James TM et al (2002).

Recent studies on Pyoderma indicate Staphylococcus aureus to be the main causative agent which is a significant change in the microbial trend of pyoderma. Previously Streptococcus pyogenes was the common etiological agent isolated. Moreover affinity of S.aureus to all mammalian cells including keratinocytes is shown in a study by Katarina Chiller et al[32] (2001).

The teichoic acid in the cell wall of S.aureus is responsible for attachment to epithelial cells and binding with fibronectin. Another important factor is nasal carriage of S.aureus which is more prevalent among children in thickly populated areas. This factor is highlighted in studies conducted by Veena Shetty et al[48] (2014) on Skin microflora and Bacterial infections of the skin.

Malnutrition may adversely affect the immune status of these children of low socioeconomic group which is conducive for establishment of infection by S.aureus as proven by Cole et al (1986). Most of our patients were from urban slum living in thickly populated areas where children are constantly exposed to insect bite and minor trauma, all of which increase S.aureus skin colonisation and cause disease.

S.pyogenes were isolated only from few of the primary pyoderma cases. This could be due to secondary invasion by S.aureus which produces

bacteriocins toxic to streptococcus pyogenes, that inhibit their growth and due to bacterial interference as stated by Paudel U et al[6] (2013).

Enterococcus faecalis isolates were very minimal (1%). Since they are part of normal faecal flora, those isolated could be due to contamination of the lesion as a result of poor personal hygiene, improper hand washing,etc.

There were no Gram negative bacilli isolated as shown by Neirita Hazarika et al[5] (2012) and Paudel U et al[6] (2013). This is because this study involved only OP and not IP patients. More than 75% of pyoderma in our study was Primary pyoderma, with Impetigo as predominant lesion followed by folliculitis,furunculosis and ecthyma where S.aureus is the most common causative agent followed by S.pyogenes. And also these patients did not show any immunocompromised state. There was also no history of previous hospitalisation. All these factors might be the reason for not reporting GNB in our study.

9.5% of cases in our study were culture negative and this correlates well with studies by Shashi Gandhi et al[7] (2012) and Janardhan B et al[26] (2015). This can be attributed to the fact that patients might have had prior antibiotics or the lesions could have been at a resolving stage when the samples were collected.

In our study Staphylococcus aureus showed high sensitivity 100% for Vancomycin, Linezolid, Teicoplanin, Mupirocin and more than 90% to Doxycycline and Cefoxitin.). Maximum resistance to Penicillin (99%) and

variying degrees of resistance to Cotrimoxazole, Amoxicillin, Erythromycin, Ciprofloxacin and Cephalexin were seen. The antibiotic susceptibility pattern of our study was similar to Shireen Furtado et al[25](2014), Suresh K Malhothra et al[10] (2012).

Most Staphylococcus aureus possess mobile genetic elements (MGE) like transposons and pathogenicity islands which harbour virulence and resistance genes. Maximum resistance to penicillin is due to penicillinase encoded by bla gene carried on the plasmid prevalent both in community and hospital acquired S.aureus strains. In addition methicillin resistance in certain strains is due to presence of pathogenicity island Staphylococcal chromosome cassette (SCC)mec, that contains chromosomal gene mecA which encodes for penicillin binding protein 2A (PBP2A).

In our present study S.pyogenes were 100% sensitive to Penicillin, Bacitrcin, Clindamycin, Doxycycline, Linezolid, Vancomycin and Mupirocin. Least sensitivity was shown by Erythromycin with 71.5%. Suresh K Malhotra et al[10] (2014) study shows similar antibiotic sensitivity pattern. Erythromycin was mostly used as an alternative to penicillin. Overusage of Erythromycin could be the reason for emergence of resistance.

Two isolates of E.faecalis 1% were recorded in our study. These were 100% sensitive to Ciprofloxacin, Doxycycline, Linezolid, Vancomycin and High level gentamycin, 50% sensitive to Erythromycin and Ampicillin. This corresponds to the study of Paudel U et al[6] (2013) whereas Janardhan B et al[26] (2015) reported all isolates to be sensitive to Erythromycin.

In our study we had isolated about 7.8% (n=12) of CA-MRSA strains. Similar findings were reported by. Kacou-N douba et al[37] (2011) which shows 14.8%, Shireen Furtado et al[25] (2014) recorded 11.3%, and Nagaraja et al[55] (2004) 11.8% .However lower prevalence rates were reported by Rahul Patil et al[60] (2006) in Mumbai as 1.4%. Parasa L S Kumar et al[65] (2010) from Andhra reported nil CA-MRSA strains.

Gureng Shrijana et al[17] (2012) and Dr Jayavardhana et al [16](2014) have recorded 48.14% and 35% of CA-MRSA, contradictory to our findings. The high prevalence may be because their study was carried out in a corporate hospital where patients from high socioeconomic group who are exposed to higher antibiotics even for minor ailments attend. Also their study included all samples from both IP and OP patients without disease specification in contrast to our study that included only paediatric cases with pyoderma attending dermatology OPD. Almost all patients in our study did not have exposure to high level antibiotics such as fluroquinolones routinely.

Most of the CA-MRSA strains isolated in our study were among 1-5 years of age (66%). Similar finding was observed by Martha Helana Von Specht et al[52] (2014) who showed correlation between CA-MRSA colonisation of nares and Skin and soft tissue infections (SSTIs) in children. Nasal carriage of CA-MRSA strain is also found to be one of the reasons for the prevalence of CA-MRSA as shown by Maureen et al[54] (2014), Veena Shetty et al[48] (2014). But the prevalence may vary. Low prevalence

in children below 1 year may be due to children remaining indoors because of close bonding with their parents and hence they do not mingle in the community on their own. Older children were also affected due to overcrowding and poor personal hygiene and close contact with family members and parents.

CA-MRSA strains are usually associated with Skin and Soft tissue infection rather than other systems. Michael Z David et al[38] (2010) and Dr Abdul Gafoor et al[15] (2012) showed these strains mainly cause SSTIs. In our study CA-MRSA strains were frequently associated with folliculitis (58%) and this is similar to the study by Cathy I.Cheng et al[69] (2008). Whereas Ralte Lalremruata et al[66] (2014), Martin E Strejewski et al [68](2008) and Martha Helana Van Specht et al[52] (2014) have shown abscesses as the commonest clinical manifestation encountered in CA-MRSA infection. Studies of Nadia Liassine et al[51] (2004), B V S Krishna et al[18] (2004) have shown furuncles to be the common lesion. This indicates that the pattern of lesion varies region wise.

Children may be at a greater risk of infection by CA-MRSA isolates having SCC mec type IV gene. PVL gene has also been universally detected among CA-MRSA strains causing SSTIs and has a strong epidemiological link with CA-MRSA isolates.

Genotyping by PCR was done for all CA-MRSA strains to identify pvl genes and also smaller SCC mec elements. CA-MRSA strains isolated in our study showed 100% positivity for mecA gene and 83% positivity

for pvl gene. This finding was supported in a Study by Nadia Lissine et al[51] (2004) were pvl gene positivity was 70%. Similarly an observation by Martha Helana Van Specht et al[52] (2014) at a paediatric hospital in Argentina has stated that all such isolates were mecA positive while only 62% were pvl positive. Specimen collection protocol should be followed strictly for gene study because contamination by skin flora might result in low pvl positivity.

Increased use of Fluoroquinolones have also been documented to increase colonisation by MRSA strains thereby increasing beta lactam resistance. The mechanism is due to elimination of MSSA strain from nasal mucosa which predispose dissemination of both MRSA and CA-MRSA strains.

The CA-MRSA strains isolated in our centre were 100% susceptible to Doxycycline, Linezolid, Vancomycin, Teicoplanin and Mupirocin, 83.3% sensitive to Clindamycin and 33% to Gentamycin and Cotrimoxazole. Similar observations were made by Dr Jayavardhana A et al[16] (2014) and Neeraj Goel et al[39] (2010) and C Bouchiat et al (2015). Ameer Abbas et al[71] (2015) and Gureng Shrijana et al[17] (2012) showed 17% and 16% sensitivity of CA-MRSA strains to cotrimoxazole which seems to be low due to indiscriminate use and easy access to this drug in district hospital, primary health centres and Balwadis in our country.

Inducible Clindamycin resistance among CA-MRSA isolates was 16.5% in our study, since the presence of class A mec complex alone

was prevalent as indicated by Molecular methods. Our finding was similar to M S Shenoy et al[44] (2010) study which shows 15.65% and that of Gureng Shrijana et al[17] (2012) shows 7.6%.

Mupirocin resistance was studied among Staphylococcus aureus and Streptococcus pyogenes isolates of our study. All our isolates were susceptible to Mupirocin(100%) mainly because topical mupirocin ointment is rarely used in our hospital. .Similar findings were observed by Jacob S.Houge et al[49] (2010) and Rajakumari et al[57] (2014). Whereas S K Oommen et al[47] (2010), Manohar Shoorshetty Rudresh et al[64] (2015) document 1.8% and 8.2% Mupirocin high level resistance (MuH).

Most of these uncomplicated CA-MRSA isolates are susceptible to clindamycin and since the outcome is favourable it remains the mainstay in therapy of CA-MRSA skin infections. Oral therapy with doxycycline and linezolid are also effective, but high cost of linezolid has limited its use.

SUMMARY

SUMMARY

- In our study 200 children less than 12 years of age with pyoderma were included.
- Among them male children were more predominant than female.
- Most common age group affected were preschool children (1-5year).
- Increased incidence of pyodermal cases noted in the month of April and May shows climatic influence over pyoderma.
- Primary pyodermal lesions were 75% and constituted major type of pyoderma.
- Impetigo was the predominant skin lesion among pyoderma and within primary pyodermal cases 53%.
- Scabies with secondary infection 45% was the commonest among secondary pyoderma.
- Lower limb was the commonest site involved in these pyodermal lesions
- Children from low socioeconomic group were the most affected 93.5%.
- Staphylococcus aureus as the predominant organism 76% followed by Streptococcus pyogenes 7% shows the change in trend of isolates.
- S,aureus showed high resistance to penicillin followed by resistance to frequently used antibiotics like Cotrimoxazole, Amoxicillin,

Erythromycin, Ciprofloxacin and cephalexin and this is of considerable importance in formulating an antibiotic policy.

- Streptococcus pyogenes were 100% sensitive to Penicillin but Erythromycin resitance among this strain is observed.
- Inducible Clindamycin resistance was 15% among MSSA and 16.5% in CA-MRSA strains.
- Constitutive Clindamycin resistance 4% was seen only among MSSA strains.
- The twelve isolates of CA-MRSA strains genotypically confirmed in our study, showed marked prevalence among low socioeconomic group in our area.

CONCLUSION

CONCLUSION

Our study shows Impetigo as the most common pyodermal lesion. Most of them affected were of the preschool age group (1-5yrs). Staphylococcus aureus was the predominant isolate followed by Streptococcus pyogenes and Enterococci was the least. S.aureus was highly sensitive to Doxycyline. Resistance to Cotrimoxazole, Amoxicillin, Erythromycin and Ciprofloxacin were significant. Most of the strains were found to be resistant to one or more antibiotics.

CA-MRSA have been implicated in 75% of community associated SSTIs in children. The reason for emergence of this strain was attributed to over usage of antibiotics, poor personal hygiene and overcrowding. This strain gets transmitted by close contact and sharing of fomites.

In most instances dermatologist prefer empirical treatment. Multidrug resistance has become a clinical challenge. Our study clearly shows the change in trend of isolates, antibiotic resistance pattern among them and prevalence of CA-MRSA in our setup. In this context clinicians should be aware of varied presentation of CA-MRSA strains and refer pyoderma cases to Diagnostic Microbiology Laboratory for culture and sensitivity so that better treatment options and spread of resistant strains in the community may be prevented. Hence implementation of appropirate antibiotic policy is need of the hour.

An MRSA control programme must be started which include education for proper hand washing, judicious use of antibiotics and increase surveillance for identification of carriers among health workers and preventive measures to reduce transmission.

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ANNEXURES

CONSENT FORM

You, _____, aged ____ years, S/o / D/o / _____, residing at _____ _____ are requested to be a participant in the research study titled *"Bacteriological Profile of PYODERMA in Paediatric age group (0-12 years) attending Dermatology OPD in Tertiary care hospital, South India"* conducted by Dr.J.Pandian, one of the post graduate trainees in the Dept. of Microbiology, Govt. Coimbatore Medical College and Hospital, Coimbatore. You are eligible for the study as per the inclusion criteria. You can ask him any question or seek from him any clarifications about the study which you may have before agreeing to participate in the study.

STATEMENT OF CONSENT

I, Father/Mother/Guardian of _____, do hereby volunteer and consent to participate my ward in this study being conducted by Dr.J.Pandian. I have read and understood the consent form (or) it has been read and explained to me thoroughly. I am fully aware of the study details as well as aware that I may ask questions to him at any time regarding this research on my ward.

Signature / Left Thumb Impression of the Father / Mother / Guardian

Station: Coimbatore

Date:

Signature / Left Thumb Impressionand Name of the witness

Station: Coimbatore

Date:

PROFORMA

Name: Date:

Age: Sex:

Lab no:

Occupation of parent/Guardian:

Income:

Address:

Presenting Complaints:

H/O of previous treatment : yes/no

H/O of previous hospitalization within 1 year : yes/no

H/O of I V antibiotics within 1 year : yes/no

H/O of similar illness in past : yes/no

H/O of similar illness for family members : yes/no

Site of Involvement:

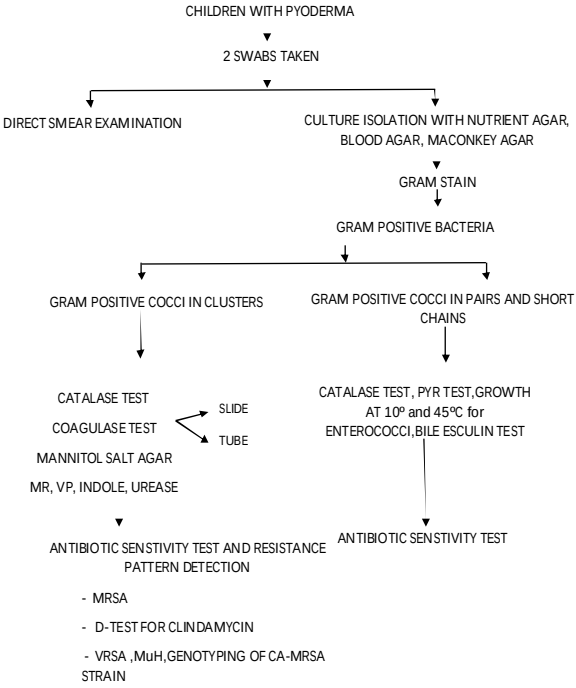
Morphology of lesion:

Risk factors

Investigations:

Diagnosis:

WORK SHEET


CHILDREN WITH PYODERMA
2 SWABS TAKEN
DIRECT SMEAR EXAMINATION
CULTURE ISOLATION WITH NUTRIENT AGAR, BLOOD AGAR, MACONKEY AGAR
GRAM STAIN
GRAM POSITIVE BACTERIA
GRAM POSITIVE COCCI IN CLUSTERS
GRAM POSITIVE COCCI IN PAIRS AND SHORT CHAINS
CATALASE TEST
COAGULASE TEST
SLIDE
TUBE
MANNITOL SALT AGAR
MR, VP, INDOLE, UREASE
CATALASE TEST, PYR TEST,GROWTH AT 10º and 45ºC for ENTEROCOCCI,BILE ESCULIN TEST
ANTIBIOTIC SENSTIVITY TEST AND RESISTANCE PATTERN DETECTION
ANTIBIOTIC SENSTIVITY TEST
- MRSA
- D-TEST FOR CLINDAMYCIN
- VRSA ,MuH,GENOTYPING OF CA-MRSA STRAIN

MASTER CHART

MATRIX CHART														
Sha	Age			Sex	Economy Group	Diagnosis	Primary / Secondary	Stress Reaction	Long-term Outcome	Single or Polymyxobial	AST			Resistance Pattern Maternal	
	<1 Mo	1 Mo–1 Yr	1–5	5–12							Sensitive	Resistant	D.F.C		
1	-	Y	–	-	M	Poor	Impetigo	Pri	Thick	Survives	S	Any: Gen, Inv, Cli, E. Bac, Pr, Van, Is Ch, CX, Mu, P, TET	Car, Pch	01.09.1 F	–
2	Y	-	-	-	F	Poor	Leptospirosis with Infarct	Sec	Thick	Survives	S	Car, Gth, Clr, P, Ba, Van, Is Ch, CI, Mu, P, TET, ATM, Cli, F	Pch, Any	01.09.1 F	–
3	-	-	Y	-	M	Poor	Cellulitis with Sac Infarct	Sec	Ul, Any Chest wall	Survives	S	Car, F, Cli, ATM, Ba, Van, Is Ch, CX, Mu, P, TET	Pch, Gth, Clr, Any	09.09.1 F	–
4	-	-	-	Y	F	Poor	Cellulitis with Sac Infarct	Sec	Ul, Il, Trunk	Survives	S	Any: Gth, Clr, F, Cli, ATM, Ba, Van, Is Ch, CX, Mu, P, TET	Pch, Car	09.09.1 F	–
5	-	-	-	Y	F	Poor	Impetigo	Pri	Chrisalosis	Survives	S	Any: Cot, Clr, F, Cli, ATM, Ba, Van, Is Ch, CX, Mu, P, TET	Pch, Gth	09.09.1 F	–
6	-	-	-	Y	F	Poor	Cellulitis	Pri	Il	Survives	S	Car, Gth, Clr, F, Cli, ATM, Ba, Van, Is Ch, CX, Mu, P, TET	Pch, Any	09.09.1 F	–
7	-	-	Y	-	F	Poor	Impetigo	Pri	Barthil	Survives	S	Car, F, Cli, Ba, Van, Is Ch, CX, Mu, P, TET	Pch, Gth, Clr, ATM, Ch, Any	09.09.1 F	–
8	-	-	Y	-	F	Poor	Impetigo	Pri	Right leg	Survives	S	Any, Gth, Clr, P, Ba, Van, Is Ch, CX, Mu, P, TET, COT, F, Cli, ATM	Pch	09.09.1 F	–
9	-	-	-	Y	F	Poor	Impetigo	Pri	Thick	Survives, Smaps progress	P	Any: Gth, Clr, F, Cli, ATM, Ba, Van, Is Ch, CX, Mu, P, TET	Car, Pch	09.09.1 F	–
-	-	-	-	-	-	-	-	-	-	Pan, B, CIP, Cli, Ba, Van, Is Gr, Mu, P	-	Car	-	-	-
10	-	-	Y	-	M	Poor	Air bleb with sac Infarct	Sec	Ul, Il	Survives	S	Gth, CIP, Cli, ATM, Van, Is CX, Mu, P, TET	Car, Pch, DO, Ch, Any	09.09.1 F	–
11	-	Y	-	-	M	Poor	Impetigo	Pri	Barthil	Survives	S	Car, Gth, Clr, F, Cli, ATM, Ba, Ch, Van, Is CX, Mu, P, TET	Pch, Any	17.09.1 F	–
12	-	-	Y	-	M	Poor	Cellulitis with Sac Infarct	Sec	Ul, Abdomen	Survives	S	Any: Gth, Clr, Ba, Van, Is Ch, CX, Mu, P, TET	Car, Pan, F, Cli, ATM	22.09.1 F	Ind Cli, Clr, F, Cli, ATM, Ch, Any, Van, Is, Ch, Is, Cl
13	-	-	Y	-	F	Poor	Cellulitis	Pri	Right forearm	Survives	S	Gth, F, Cli, ATM, Ba, Van, Is Ch, Mu, P, TET	Pch, COT, CIP, Any	22.09.1 F	–
14	-	-	Y	-	F	Poor	Bullous impetigo	Pri	Right forearm	Survives	S	Any: ATM, F, Cli, Clr, CIP, Ba, Van, Is Ch, CX, Mu, P, TET	Pan, Car	01.10.1 F	–
15	-	-	-	Y	F	Poor	Cellulitis with Sac Infarct	Sec	Ul, Trunk	Survives	S	Any, Gth, Clr, F, Ba, Van, Is CX, Mu, P, TET	Car, Pan, ATM, Cli, Ch	29.10.1 F	–
16	-	Y	-	-	M	Poor	Bullous impetigo	Pri	Stomach	Survives	S	Any, Car, Gth, Cli, ATM, CIP, Ba, Van, Is Ch, CX, Mu, P, TET	Pan, F	29.10.1 F	–
17	-	-	-	Y	M	Poor	Empyema thoracis	Sec	Urethrovia	Survives	S	Any: Gth, Pch, F, Cli, ATM, CIP, Ba, Van, Is Ch, CX, Mu, P, TET	Car	29.10.1 F	–
18	-	-	-	Y	M	Poor	Air bleb with sac Infarct	Sec	Ul	Survives	S	Any, Gth, Clr, F, Cli, ATM, Ba, Van, Is Ch, CX, Mu, P, TET	Car, Pan	29.10.1 F	–
19	-	-	Y	-	M	Poor	Erythrova	Pri	Il	Streptococcus	S	Pan, B, F, Cli, PCB, Ba, Van, Is Gr, Mu, P, ATM	Car, Gth	29.10.1 F	–
20	-	Y	-	-	M	Poor	Impetigo	Pri	Right axillabris	-	-	-	-	31.10.1 F	–
21	-	-	Y	-	M	Poor	Impetigo	Pri	Barthil	Survives	S	COT, Gth, Clr, F, Cli, ATM, Ba, Van, Is Ch, CX, Mu, P, TET	Pan, Any	19.11.1 F	–
22	-	-	Y	-	M	Poor	Impetigo	Pri	Barthil	Survives	S	Any, Gth, Clr, F, Cli, ATM, Ba, Van, Is Ch, CX, Mu, P, TET	Car, Pan	19.11.1 F	–
23	-	-	Y	-	M	Poor	Pneumolasis	Pri	Barthil	Survives	S	Car, Gth, Clr, F, Cli, ATM, Ba, Van, Is Ch, CX, Mu, P, TET	Any, Pan	17.11.1 F	–
24	-	-	Y	-	M	Poor	Impetigo	Pri	Scalp	Survives	S	Any, Gth, Clr, F, Cli, ATM, Ba, COT, Van, Is Ch, CX, Mu, P, TET	Pan	19.11.1 F	–
25	-	-	Y	-	F	Poor	Impetigo	Pri	Stomach	Grows, smaps progress	P	Any: Gth, Clr, F, Cli, ATM, Ba, Van, Is Ch, CX, Mu, P, TET	Car, Pan	19.11.1 F	–
-	-	-	-	-	-	-	-	-	-	Pan, B, F, Cli, PCB, Ba, Van, Is Gr, Mu, P, ATM	-	-	-	-	-
26	-	-	Y	-	F	Poor	Cellulitis with Sac Infarct	Sec	Ul, Il	Survives	S	Car, Gth, Clr, F, Cli, ATM, Ba, Van, Is Ch, CX, Mu, P, TET	Any, Pan	21.11.1 F	–
27	Y	-	-	-	F	Poor	Impetigo	Pri	Jolts	Survives	S	Any, Gth, Clr, F, Cli, ATM, Ba, Van, Is Ch, CX, Mu, P, TET	Car, Pan	21.11.1 F	–
28	-	-	Y	-	M	Poor	Cellulitis	Pri	Barthil	Survives	S	Car, F, Cli, Ch, CIP, Ba, Van, Is Mu, P, TET	Gth, Cx, CIP, ATM, Pch	25.11.1 F	Ch-Jol(S)
29	-	-	Y	-	M	Poor	Pneumolasis	Pri	Barthil	Survives	S	Any, Gth, Clr, F, Cli, ATM, Ba, Van, Is Ch, CX, Mu, P, TET	Pan, Car	25.11.1 F	–
30	-	-	Y	-	M	Poor	Cellulitis with Sac Infarct	Sec	Barthil	Survives	S	Any, Gth, Clr, F, Cli, ATM, Ba, Van, Is Ch, CX, Mu, P, TET	Car, Pan	25.11.1 F	–
31	-	-	-	Y	M	Poor	Impetigo	Pri	Scalp	-	-	-	-	28.11.1 F	–
32	-	-	Y	-	M	Poor	Impetigo	Pri	Ul, Trunk	Smaps progress	S	Pan, Cli, B, CIP, Ba, Van, Is Ch, Mu, P, ATM, Gth	Car, F	28.11.1 F	–
33	-	-	Y	-	M	Poor	Cellulitis with Sac Infarct	Sec	Barthil	Grows, smaps progress	P	Gth, Clr, Ch, Pch, DO, CIP, Ch, CX, Mu, P, TET	Car, Pan, F, Any	28.11.1 F	–
-	-	-	-	-	-	-	-	-	-	-	-	F, Cli, B, CIP, Ba, Van, Is Gr, Mu, P, ATM, Pan	Car	-	-
34	-	-	Y	-	F	Poor	Cellulitis with Sac Infarct	Sec	Barthil, Il	Survives	S	Car, Gth, Clr, P, ATM, Ba, Van, Is Ch, CX, Mu, P, TET	Pan, F, Cli, Any	2.12.1 F	Ind Cli, Clr, F, Cli, ATM, Ch, Any, Van, Is Ch, Is, Cl
35	Y	-	-	-	F	Poor	Impetigo	Pri	Chin	Survives	S	CIP, F, Cli, ATM, Ba, Van, Is Ch, CX, Mu, P, TET	Car, Pan, Gth, Any	2.12.1 F	–
36	-	-	Y	-	F	Poor	Cellulitis with Sac Infarct	Sec	Barthil, Il	Survives	S	Car, F, Cli, ATM, Ba, Van, Is Ch, Mu, P, TET	Gth, Cx, Ch, CIP, Pch	2.12.1 F	Ch-Jol(S)
37	Y	-	Y	-	M	Poor	Cellulitis	Pri	Knee	Survives	S	Any, Gth, F, ATM, Ba, Van, Is Ch, CX, Mu, P, TET	Pch, Car, Ch, CIP, Cli	2.12.1 F	–
38	Y	-	-	-	F	Diabetes	Impetigo	Pri	Knee	Survives	S	Any: Gth, Clr, F, Cli, ATM, Ba, Van, Is Ch, CX, Mu, P, TET	Car, Pan	2.12.1 F	–
39	-	-	Y	-	M	Poor	Impetigo	Pri	Barthil	Survives	S	Any, Clr, F, Cli, ATM, Ba, Van, Is Ch, CX, Mu, P, TET	Car, Pan, Gth	2.12.1 F	–
40	Y	-	-	-	M	Poor	Erythrova	Pri	Il	Survives	S	Gth, Clr, F, Cli, ATM, Ba, Van, Is Ch, CX, Mu, P, TET	Car, Pan, Any	2.12.1 F	–
41	-	-	Y	-	M	Poor	Impetigo	Pri	Scalp	Survives	S	Gth, F, Cli, ATM, Ba, Van, Is Ch, CX, Mu, P, TET	Pan, Clr, Car, Ch, ATM, Any	2.12.1 F	–
42	-	-	-	Y	M	Poor	Air bleb with sac Infarct	Sec	Trunk	Survives	S	Car, Gth, Clr, Cli, ATM, Ba, Van, Is Ch, CX, Mu, P, TET	Pan, F, Any	2.12.1 F	–
43	-	-	Y	-	F	Poor	Cellulitis	Pri	Ul	-	-	-	-	2.12.1 F	–
44	-	-	Y	-	M	Poor	Bullous impetigo	Pri	Il	Survives	S	Any: Clr, F, Cli, Ba, Ch, ATM, Van, Is Ch, CX, Mu, P, TET	Car, Pan, Gth	2.12.1 F	–
45	-	-	-	Y	M	Poor	Cellulitis	Pri	Ul	Survives	S	Clr, F, Cli, ATM, Van, Is Ch, Mu, P, TET	Gth, Car, Cx, Ch, Pan	05.01.15	Ch-Jol(S)
46	-	-	-	Y	M	Poor	Cellulitis	Pri	Ul	Survives	S	Any, Clr, F, Cli, Ba, ATM, Van, Is Ch, CX, Mu, P, TET	Car, Pan, Gth	05.01.15	–
47	-	-	Y	-	M	Poor	Cellulitis with Sac Infarct	Sec	Barthil, Il	Survives	S	Car, Gth, Clr, Cli, ATM, Ba, Van, Is Ch, CX, Mu, P, TET	Any, Pan, F	05.01.15	–
48	-	-	Y	-	F	Gangrene	Impetigo	Pri	Stomach	Survives	S	Any, Cot, Clr, F, Cli, ATM, Ba, Van, Is Ch, CX, Mu, P, TET	Pan, Gth	05.01.15	Ch-Jol(S)
49	-	-	Y	-	F	Poor	Cellulitis	Pri	Scalp	Survives	S	F, Cli, ATM, Ba, Van, Is Ch, Mu, P, TET	Pan, Car, Cx, Clr, Gth	05.01.15	Ch-Jol(S)
50	-	-	-	Y	M	Poor	Impetigo	Pri	Il	Survives	S	Gth, F, ATM, Ba, Van, Is Ch, CX, Mu, P, TET	Any, Car, Pan, Clr, Cli, Ch	05.01.15	–
51	-	-	-	Y	M	Diabetes	Cellulitis with Sac Infarct	Sec	Il	Grows, smaps progress	P	Gth, Clr, F, Cli, ATM, Ba, Van, Is Ch, CX, Mu, P, TET	Any, Car, Pan	05.01.15	–
-	-	-	-	-	-	-	-	-	-	Pan, F, Cli, B, CIP, Ba, Van, Is Gr, Mu, P, ATM	-	Car	-	-	-
52	-	-	Y	-	M	Poor	Impetigo	Pri	Il	Survives	S	CIP, ATM, Ba, Van, Is Ch, CX, Mu, P, TET	Any, Car, Pan, F, Gth, Cli, Ch	07.01.15	Ind Cli, Clr, F, Cli, ATM, Ch, Any, Van, Is Ch, Is, Cl
53	Y	-	-	-	M	Diabetes	Impetigo	Pri	Il	Survives	S	Any, Gth, Pch, Clr, F, Cli, ATM, Ba, Van, Is Ch, CX, Mu, P, TET	Car	07.01.15	–

54	-	-	Y	-	M	Poor	Confirms	Prt	II	Spawns	S	Any; CON CP E CD, ATM (Ba Vent) > Ch CX MuP, TEI	Pen GEh	07.01.15		-	
55	-	-	-	Y	M	Poor	Pancreatitis	Prt	II	Spawns	S	Car GEh E CD ATM (Ba Vent) > Ch CX MuP, TEI	Pen CP Eh Any;	08.01.15		-	
56	-	-	-	Y	M	Poor	Call dirts	Prt	II	Strep progenies	S	Pen B CP CD (Ba Vent) > MuP ATM Cyr GEh	Car E	08.01.15		-	
57	-	-	-	Y	M	Poor	Follicul its	Prt	Flora	Spawns	S	Any; GEh CP E CD ATM (Ba Vent) > Ch CX MuP, TEI	Car Pen	08.01.15		-	
58	-	Y	-	-	M	Poor	Impetigo	Prt	Cor labella	-	-	-	-	08.01.15		-	
59	-	-	-	Y	M	Poor	Amyotrophic Lateral Sclerosis	Sac	Ly II	Spawns	S	Car CP Ba Vent) > MuP, TEI	Pen CX Ch E CD GEh ATM	10.01.15	CX-AMSA	INDUCIBLE CD INDUCIBLE CD INDUCIBLE CD	RESISTANCE
60	Y	-	-	-	M	Poor	Impetigo	Prt	Cor labella	Strep progenies	S	Pen CP B E CD ATM (Ba Vent) > MuP GEh	Car	10.01.15		-	
61	-	Y	Y	-	M	Poor	Follicul its	Prt	II	Spawns	S	E CD, ATM (Ba Vent) > Ch CX MuP, TEI	Any; Pen CP GEh Car	12.01.15		-	
62	-	-	Y	-	M	Poor	Erythema	Prt	II	Spawns	S	GEh CP E CD, ATM (Ba Vent) > Ch CX MuP, TEI	Any; Car Pen	20.01.15		-	
63	-	-	Y	-	F	Poor	Atrial fibrillation	Sac	II	Spawns	S	Any; GEh CP, ATM (Ba Vent) > Ch, CX MuP, TEI	Car Pen E, CD EO	20.01.15		INDUCIBLE CD INDUCIBLE CD INDUCIBLE CD	RESISTANCE
64	-	Y	-	-	M	Poor	Follicul its	Prt	II	Spawns	S	GEh, CP, CD E, CD, ATM (Ba Vent) > Ch, CX MuP, TEI	Any; Pen	21.01.15		-	
65	-	-	-	M	Poor	Granulomatosis with Polyangiitis	Prt	Impetigo	II	Spawns	S	Any; Car E, CD ATM (Ba Vent) > Ch, CX MuP, TEI	Pen CP, GEh	21.01.15		-	
66	-	-	Y	-	M	Poor	Atrial fibrillation	Sac	II	Spawns	S	Any; CP GEh, ATM (Ba Vent) > Ch CX MuP, TEI	Car Pen E CD	21.01.15		INDUCIBLE CD INDUCIBLE CD INDUCIBLE CD	RESISTANCE
67	-	-	Y	-	M	Poor	Impetigo	Prt	II	Strep progenies	S	Pen GEh, B CP, E CD (Ba Vent) > ATM MuP, Cyr	Car	22.01.15		-	
68	-	-	Y	-	M	Poor	Follicul its	Prt	Trunk	Spawns	S	Car GEh CP E CD, ATM (Ba Vent) > Ch CX MuP, TEI	Any; Pen	22.01.15		-	
69	-	-	-	-	M	Poor	Follicul its	Prt	II	Spawns	S	Car GEh E CD, ATM (Ba Vent) > Ch CX MuP, TEI	Any; Pen	25.01.15		-	
70	-	-	-	Y	F	Poor	Clerical blockage with Impacted Cerumen	Sac	Ly II	Spawns	S	Any; CP E CD, ATM (Ba Vent) > MuP, TEI	Car Pen GEh Ch	25.01.15		-	
71	-	Y	-	-	F	Poor	Furunculosis	Prt	II	-	-	-	-	27.01.15		-	
72	-	-	Y	-	M	Diabetes	Paronychia	Prt	Fingernail	Spawns	S	Any; GEh, CP E, CD, ATM (Ba Vent) > Ch, CX MuP, TEI	Car Pen	27.01.15		-	
73	-	-	Y	-	M	Poor	Cellulitis	Prt	Scalp	Spawns	S	Any; Car GEh, CP, B, ATM (Ven) > Ch CX MuP, TEI	Pen, CD	05.02.15		INDUCIBLE CD INDUCIBLE CD INDUCIBLE CD	RESISTANCE
74	-	-	-	-	F	Poor	Erythema	Prt	II	Spawns	S	Car GEh, CD, ATM (Ba Vent) > Ch CX MuP, TEI	Pen, D Any	05.02.15		-	
75	-	-	Y	-	F	Poor	Impetigo	Prt	II	Spawns	S	GEh CD, ATM (Ba Vent) > Ch CX MuP, TEI	Car Pen, E CP Any;	05.02.15		-	
76	-	-	Y	-	F	Poor	Cellulitis	Prt	II	Enterococci	S	Any; MG, CP (Ba Vent) > TEI	Pen E	05.02.15		-	
77	-	-	Y	-	M	Poor	Erythema	Prt	II	-	-	-	-	06.02.15		-	
78	-	-	Y	-	M	Poor	Atrial fibrillation with Impacted Cerumen	Sac	Ly II	Spawns	S	Any; GEh, E CD, ATM (Ba Vent) > CX MuP, TEI	Car Pen, CP Ch	06.02.15		-	
79	-	-	-	Y	M	Poor	Acute Myeloid Leukemia	Sac	II	-	-	-	-	06.02.15		-	
80	-	-	Y	-	F	Poor	Impetigo	Prt	Stomach	Spawns	S	E, ATM, CP CD (Ba Vent) > Ch CX MuP, TEI	Any; GEh, Pen Car	06.02.15		-	
81	Y	-	-	-	F	Poor	Impetigo	Prt	Chin	Strep progenies	S	Pen GEh, B CP CD (Ba Vent) > ATM MuP Cyr	Car E	09.02.15		-	
82	-	-	Y	-	M	Poor	Furunculosis	Prt	Face	Spawns	S	Car GEh, CP, CD, ATM (Ba Vent) > Ch CX MuP, TEI	Any; Pen E	09.02.15		-	
83	-	-	-	Y	F	Poor	Atrial fibrillation with Impacted Cerumen	Sac	Ly, Trunk II	Spawns	S	Any; GEh, CD, ATM (Ba Vent) > CX MuP, TEI	Car Pen, E CP Ch	09.02.15		-	
84	-	-	-	Y	F	Poor	Granulomatosis with Polyangiitis	Prt	Fingernail	Spawns	S	GEh CP, E CD, ATM (Ba Vent) > Ch CX MuP, TEI	Any; Car Pen	09.02.15		-	
85	-	-	Y	-	M	Poor	Cellulitis	Prt	II	Spawns	S	Car GEh, CP, E CD, ATM, Ch (Ba Vent) > MuP, TEI	Pen CX	13.02.15		CX-AMSA	
86	-	-	Y	-	M	Poor	Impetigo	Prt	II	Spawns	S	Any; Car GEh, ATM (Ba Vent) > Ch, CX MuP, TEI	Pen E, ATM GEh, Ch	13.02.15		-	
87	-	Y	-	-	F	Poor	Erythema	Prt	II	Strep progenies	S	Pen B, E, CP CD (Ba Vent) > ATM MuP, Cyr	Car GEh	13.02.15		-	
88	-	-	-	Y	F	Poor	Impetigo	Prt	Scalp	Spawns	S	Car (Ba Vent) > Ch, CX MuP, TEI	Any; Pen, E GEh, CP, ATM	13.02.15		-	
89	-	-	-	-	M	Poor	Impetigo	Prt	Nares Streptococci	Strep progenies	P	GEh, CP E, CD, ATM (Ven) > Ch CX MuP, TEI	Any; Car Pen	17.02.15		-	
	-	-	-	-	-	-	-	-	-	-	-	Pen B CP, E CD (Ba Vent) > ATM MuP, Cyr	Car	-		-	
90	-	-	Y	-	M	Poor	Follicul its	Prt	II	Spawns	S	Car CP E CD, ATM (Ba Vent) > Ch MuP, TEI	GEh Pen CX	17.02.15		CX-AMSA	
91	-	-	-	-	Y	F	Poor	Diabetes with Acute Osteomyelitis	Sac	Ly II	-	-	-	-	20.02.15		-
92	-	-	-	-	Y	F	Poor	Impetigo	Prt	Ly II	Strep progenies	S	Pen B CP CD (Ba Vent) > ATM MuP Cyr	Car E	20.02.15		-
93	Y	-	-	-	M	Poor	Conjunctivitis	Prt	Urethra	Spawns	S	Any; GEh, E CD ATM (Ba Vent) > Ch CX MuP, TEI	Pen CP Car	09.02.15		-	
94	-	-	Y	-	M	Poor	Eczema herpeticum	Sac	Ly II	Spawns	S	Car GEh CP E CD, ATM Ch (Ba Vent) > MuP, TEI	Any; Pen	09.02.15		-	
95	-	-	Y	-	F	Poor	Impetigo	Prt	II	Spawns	S	GEh E Car CD, ATM (Ba Vent) > CX MuP, TEI	Any; Pen CP Ch	09.02.15		-	
96	Y	-	-	-	M	Poor	Impetigo	Prt	Face	Strep progenies	S	Pen B E CD (Ba Vent) > ATM MuP Cyr	Car CP	09.02.15		-	
97	-	Y	-	-	M	Poor	Atrial fibrillation with Impacted Cerumen	Sac	Ly II	Spawns	S	Any; Car GEh CP E CD, ATM (Ba Vent) > CX MuP, TEI	Pen Ch E CD	10.02.15		INDUCIBLE CD INDUCIBLE CD INDUCIBLE CD	RESISTANCE
98	-	-	Y	-	M	Poor	Follicul its	Prt	Ly II	Spawns	S	Car E, CD ATM CP GEh (Ba Vent) > Ch MuP, TEI	CX Pen	10.02.15	CX-AMSA		
99	-	-	Y	-	M	Poor	Bronchiectasis with Infection	Sac	Ly II	-	-	-	-	11.02.15		-	
100	-	-	Y	-	-	F	Poor	Cellulitis	Prt	II	Spawns	S	Any; Car E CD (Ba Vent) > Ch CX MuP, TEI	Pen CP ATM GEh	11.02.15		-
101	-	-	-	Y	-	M	Poor	Impetigo	Prt	II	Spawns	S	Any; GEh, CP E CD ATM (Ba Vent) > Ch CX MuP, TEI	Car Pen	16.02.15		-
102	-	-	-	Y	-	M	Poor	Furunculosis	Prt	Ly II	Spawns	S	Car, ATM (Ba Vent) > Ch, CX MuP, TEI	Any; Pen, E CD CP GEh Ch	16.02.15		INDUCIBLE CD INDUCIBLE CD INDUCIBLE CD	RESISTANCE
103	-	-	-	Y	-	F	Poor	Cellulitis	Prt	II	Spawns	S	Car GEh CP E CD, ATM (Ba Vent) > Ch CX MuP, TEI	Any; Pen	19.02.15		-
104	-	-	-	Y	-	F	Poor	Impetigo	Prt	II	Spawns	S	Car CP GEh, ATM Ch (Ba Vent) > CX MuP, TEI	Any; Pen E CD	19.02.15		INDUCIBLE CD INDUCIBLE CD INDUCIBLE CD
105	-	Y	-	-	M	Poor	Furunculosis	Prt	Stomach	Spawns	S	Any; Car E, CD, ATM (Ba Vent) > Ch, CX MuP, TEI	Pen CP, GEh	23.02.15		-	
106	-	Y	-	-	M	Poor	Impetigo	Prt	II	Spawns	S	Any; Car GEh, CP, CD, B O, IT, ATM Ch, CX MuP, TEI	Pen E, ATM	23.02.15		-	
107	-	Y	-	-	M	Poor	Impetigo	Prt	Chin	Strep progenies	S	Pen B CP, E CD (Ba Vent) > ATM MuP, Cyr	Car	23.02.15		-	
108	-	Y	-	-	M	Poor	Acute Myeloid Leukemia	Sac	Ly II	Nares Streptococci	P	GEh, CP, Car CD, ATM (Ba Vent) > Ch CX MuP, TEI	Any; Pen E	25.02.15		-	
	-	-	-	-	-	-	-	-	-	-	-	Pen B CP, E CD (Ba Vent) > ATM MuP, Cyr	Car	-		-	
109	-	-	Y	-	M	Poor	Impetigo	Prt	II	Spawns	S	Car CP E CD, ATM (Ba Vent) > Ch, CX MuP, TEI	Any; Pen GEh	25.02.15		-	
110	-	Y	-	-	M	Poor	Erythema	Prt	Ly II	Spawns	S	Any; GEh, CP E, CD, ATM (Ba Vent) > Ch, CX MuP, TEI	Car Pen	25.02.15		-	
111	-	-	-	Y	F	Poor	Impetigo	Prt	II	-	-	-	-	25.02.15		-	

112	-	Y	-	-	M	Poor	Irregular	Prt	II	Sauvaus	S	Jnvv, Car,GTh,CIP,BO,CD,.XfM,Va n Ch, CX,MuP,TFI	Part,C	07.04.15	-
113	-		Y	-	M	Poor	Folliculitis	Prt	Ul, II	Smap mopenes	S	Pam,GTh,B,F,CB Ba Van I> .XfM,MuP,Cir	Car,CIP	07.04.15	-
114	Y			-	F	Poor	Ir dermatitis-ith Inf	Sac	Trunk	Sauvaus	S	Car,F,CB (Ba)van I> Ch,CX,MuP,TFI	Jnv> Pam,XfM,GTh,CIP	10.04.15	-
115	-	Y		-	M	Poor	Ac iths-with sac Infec	Sac	Ul, II	Sauvaus	S	GTh,F,CB,XfM (Ba Van I> Ch, CX,MuP,TFI	Jnv> Car,Pam,CIP	10.04.15	-
116	-	-	Y	-	M	Poor	Irregular	Prt	II	Sauvaus	S	Car,F,CB,XfM (Ba Van I> Ch,CX,MuP,TFI	Jnv> Pam,GTh,CIP	06.04.15	-
117	-	-	Y	-	M	Poor	Irregular	Prt	II	Sauvaus	S	GTh,CIP,F,CB,BO,Ch,XfM(Van I> Ch,MuP,TFI	Jnv>, Car,Pam	06.04.15	-
118	-	-	Y	-	M	Poor	Ecthlies-virtrac(inPaet)	Sac	Ul, II	Sauvaus	S	Jnv> Car,GTh,CIP,XfM(Ba Van I> Ch,MuP,TFI	Pam,F,CB, Ch	07.04.15	InfluCBIC CIInDJMxVGh RSISThCt
119	M	-	-	-	M	Poor	Folliculitis	Prt	II	Sauvaus	S	Car (Ba Van) I> Ch, CX,MuP,TFI	Jnv>,Pam,F,CB, CIP,XfM,GTh	07.04.15	CONSTITUTVCCIInDJMVGh RSISThCt
120	Y	-	-	Y	M	Poor	Irregular	Prt	II	Hewes Snappyvoget	P	Jnv> GTh, CIP,F,CB,XfM(Ba Van) I> Ch, CX,MuP,TFI	Car,Pam	06.04.15	-
	-	-	-	-	-	-	-	-	-	-	-	Pam,BCIP,FCB,Ba(van) I> XfM,MuP,Cir	Car	-	-
121	-	Y	-	-	F	Poor	Ecthlies-virtrac(inPaet)	Sac	Ul, II	Sauvaus	S	Car,CIP,XfM,Ba Van I> Ch, CX,MuP,TFI	Jnv>,Pam,F,CB,GTh	10.04.15	InfluCBIC CIInDJMxVGh RSISThCt
122	Y	-	-	-	M	Poor	Irregular Indian	Prt	Face	Sauvaus	S	Jnv>, Car,GTh,CIP,CB,XfM,Ba Ch, Van I> CX,MuP,TFI	Car,Pam,CIP	10.04.15	-
123	-	-	-	Y	F	Poor	Ecthlies-virtrac(inPaet)	Sac	Ul, II	Sauvaus	S	Jnv>, CIP,F,CB,XfM(Ba Van) I> Ch,CX,MuP,TFI	Car,Pam,GTh	14.04.15	-
124	-	-	Y	-	F	Poor	Furunculosis	Prt	II	Sauvaus	S	-	-	14.04.15	-
125	-	-	Y	-	M	Poor	Ecthmya	Prt	Ul	-	-	-	-	14.04.15	-
126	-	-	Y	-	M	Poor	Irregular	Prt	II	Sauvaus	S	Jnv>, Car,GTh,CIP,XfM(Ba,a Van) I> Ch,MuP,TFI	Pam,F,CB	14.04.15	InfluCBIC CIInDJMxVGh RSISThCt
127	-	Y	-	-	F	Poor	Irregular	Prt	Face	Hewes Snappyvoget	P	Jnv>,GTh,CIP,F,CB,XfM(Ba Van) I> Ch, CX,MuP,TFI	Car,Pam	14.04.15	-
	-	-	-	-	-	-	-	-	-	-	-	Pam,BCIP,FCB,Ba(van) I> XfM,MuP,Cir	Car	-	-
128	-	-	-	Y	F	Poor	Irregular	Prt	Cir Lobule	-	-	-	-	20.04.15	-
129	-	-	Y	-	M	Poor	Ac iths-with sac Infec	Sac	II	Sauvaus	S	CIP,F,CB,.XfM(Ba Van I> Ch, CX,MuP,TFI	Jnv> Car,Pam,GTh	27.04.15	-
130	-	-	Y	-	M	Poor	Ir dermatitis-ith Inf	Sac	Ul, II	Sauvaus	S	GTh,CIP,F,CB,XfM(Ba Van I> Ch,CX,MuP,TFI	Jnv>,Pam,Ch,CIP,.XfM,Car	27.04.15	-
131	-	-	Y	-	M	Poor	Ir dermatitis-ith Inf	Sac	Ul, II	Sauvaus	S	Jnv>,Car,GTh(Ba Van I> Ch,CX,MuP,TFI	Pam,F,CB,CIP,.XfM	27.04.15	InfluCBIC CIInDJMxVGh RSISThCt
132	-	-	Y	-	M	Poor	Ecthlies-virtrac(inPaet)	Sac	Ul	Sauvaus	S	Car,F,CB,XfM(Ba Van I> MuP,TFI	Pam,CIP,CX,GTh,Ch	27.04.15	CX-AdBSA
133	-	-	-	Y	F	Poor	Furunculosis	Prt	II	Sauvaus	S	Jnv>,GTh,CIP,F,CB,XfM(Ba Van) I> Ch,CX,MuP,TFI	Car,Pam	27.04.15	-
134	-	-	Y	-	F	Poor	Irregular	Prt	Chin	Hewes Snappyvoget	P	GTh,CIP,F,CB,XfM(Ba Van) I> Ch,CX,MuP,TFI	Jnv> Car,Pam	27.04.15	-
	-	-	-	-	-	-	-	-	-	-	-	Pam,BCIP,FCB,Ba(van) I> XfM,MuP,Cir	Car	-	-
135	-	-	Y	-	F	Poor	Furunculosis	Prt	Ul	Sauvaus	S	Jnv> Car,F,CB,XfM(Ba Van) I> Ch, CX,MuP,TFI	Pam,CIP,GTh	28.04.15	-
136	-	-	Y	-	M	Poor	Folliculitis	Prt	Ul	-	-	-	-	29.04.15	-
137	-	-	Y	-	M	Poor	Irregular	Prt	II	Sauvaus	S	CIP,F,CB,.XfM(Ba Van) I> Ch,CX,MuP,TFI	Jnv>,Car,F,Pam,GTh	29.04.15	-
138	Y	-	-	-	F	Poor	Ac iths-with sac Infec	Sac	Ul	Sauvaus	S	Jnv>,GTh,CIP,F,CB,XfM(Ba Van) I> Ch,CX,MuP,TFI	Car,Pam	29.04.15	-
139	-	-	-	Y	F	Poor	Folliculitis	Prt	II	Smpp mopenes	S	Pam,BCIP,FCB,Ba Van) I> XfM,MuP,Cir,GTh	Car	29.04.15	-
140	-	-	Y	-	F	Poor	Ecthmya	Prt	II	Sauvaus	S	Car,GTh,CIP,FCB,Ba Ch, XfM,Van I> Ch,CX,MuP,TFI	Jnv> Pam	30.04.15	-
141	-	-	Y	-	F	Poor	Folliculitis	Prt	II	Sauvaus	S	CIP,XfM,Ba Van) I> Ch,MuP,TFI	Car,Pam,CIP,Ch,Cir,Clp	30.04.15	CX-AdBSA
142	-	-	Y	-	F	Clerk	Irregular	Prt	Face	Sauvaus	S	Jnv, Car,GTh,CIP,Ba Van) I> Ch,CX,MuP,TFI	Pam,F,CB,GTh,XfM	07.05.15	InfluCBIC CIInDJMxVGh RSISThCt
143	-	-	Y	-	F	Poor	Irregular	Prt	II	Sauvaus	S	CIP,F,CB,.XfM,Ba Van) I> Ch,CX,MuP,TFI	Jnv> Pam,GTh,Car	05.05.15	-
144	-	-	Y	-	M	Poor	Ac iths-with sac Infec	Sac	Ul	Sauvaus	S	Car,GTh,CB,.XfM,Ba Van) I> Ch,CX,MuP,TFI	Jnv> Pam,F,.CIP,CB	07.05.15	CONSTITUTVCCIInDJMVGh RSISThCt
145	-	-	Y	-	F	Ecthlia rupin	Irregular	Prt	Ul, II	-	-	-	-	07.05.15	-
146	M	-	Y	-	F	Poor	Irregular Nodular	Prt	Cir Lobule	Sauvaus	S	Jnv, CIP,F,CB,.XfM(Ba Van) I> Ch, CX,MuP,TFI	Car,Pam,GTh	06.05.15	-
147	Y	-	-	-	F	Poor	Irregular	Prt	Face	Sauvaus	S	Jnv>,XfM,Ba Ch, Van I> CX,MuP,TFI	Pam,F,CB,CIP,GTh	06.05.15	InfluCBIC CIInDJMxVGh RSISThCt
148	-	Y	-	-	M	Poor	Folliculitis	Prt	II	Sauvaus	S	Jnv>,GTh,Ba Van) I> Ch, CX,MuP,TFI	Car,Pam,F,CB,CIP,.XfM	06.05.15	InfluCBIC CIInDJMxVGh RSISThCt
149	M	-	-	-	F	Poor	Ac iths-with sac Infec	Sac	Ul, Trunk,II	Sauvaus	S	Car,GTh,.CIP,Ba Van) I> Ch,CX,MuP,TFI	Jnv> Pam,Ch,F,CIP	06.05.15	-
150	-	Y	-	-	F	Bathtub	Irregular	Prt	II	Sauvaus	S	GTh,CIP,F,CB,.XfM(Ba Van) I> Ch,CX,MuP,TFI	Jnv, Car,Pam	06.05.15	-
151	-	Y	-	-	M	Poor	Folliculitis	Prt	Scalp	Sauvaus	S	Jnv, CIP,F,CB,XfM(Ba Van) I> Ch,CX,MuP,TFI	Car,Pam,GTh	06.05.15	-
152	M	Y	-	-	M	Poor	Irregular Nodular	Prt	II	Sauvaus	S	GTh,.CIP,Ba Van) I> Ch,CX,MuP,TFI	Car,Pam,F,CX	06.05.15	CX-AdBSA
153	-	-	Y	-	M	Poor	Folliculitis	Prt	II	Sauvaus	S	Jnv>,GTh,CIP,F,CB,Ba Van) I> Ch,MuP,TFI	Car,Pam,XfM	06.05.15	-
154	-	-	Y	-	M	Poor	Ecthmya	Prt	II	Sauvaus	S	Jnv>,Car,Ba,.XfM Van) I> Ch,CX,MuP,TFI	Pam,F,CB,CIP,GTh	11.05.15	InfluCBIC CIInDJMxVGh RSISThCt
155	-	Y	-	-	M	Poor	Irregular	Prt	Face	Sauvaus	S	Jnv>,Car,GPF,CB,Ba Van) I> Ch, CX,MuP,TFI	Pam,GTh,Jnv	11.05.15	-
156	-	Y	-	-	F	Poor	Ac iths-with sac Infec	Sac	Face	-	-	-	-	11.05.15	-
157	-	-	Y	-	F	Poor	Irregular	Prt	II	Sauvaus	S	GTh,F,CB,XfM(Ba Van) I> Ch,CX,MuP,TFI	Jnv> Pam,CIP,Car	13.05.15	-
158	M	-	Y	-	M	Poor	Ecthmya	Prt	II	Smpp mopenes	S	Pam,BCIP,CB,Ba Van) I> MuP,Cir	Car,GTh	13.05.15	-
159	-	-	-	Y	M	Poor	Ecthlies-virtrac(inPaet)	Sac	Ul, II	Sauvaus	S	GTh,Ba Van) I> Ch,CX,MuP,TFI	Jnv> Car,Pam,F,CB,.CIP,.XfM	13.05.15	InfluCBIC CIInDJMxVGh RSISThCt
160	-	-	Y	-	M	Poor	Furunculosis	Prt	II	Sauvaus	S	Jnv>,Car,GTh,XfM(Ba Van) I> Ch,CX,MuP,TFI	Pam,Ch,F,CB,CIP	13.05.15	InfluCBIC CIInDJMxVGh RSISThCt
161	-	-	Y	-	F	Poor	Ac iths-with sac Infec	Sac	II	Sauvaus	S	GTh,CIP,F,CB,.XfM(Ba Van) I> Ch,CX,MuP,TFI	Jnv, Car,Pam	13.05.15	-
162	-	-	Y	-	F	Poor	Irregular Nodular	Prt	Ul, II	Sauvaus	S	GTh,CIP,F,CB,.XfM(Ba Van) I> Ch,CX,MuP,TFI	Jnv, Car,Pam	18.05.15	-
163	-	Y	-	-	M	Poor	Furunculosis	Prt	Ul	Sauvaus	S	Jnv, Car,GTh,F,CB,XfM(Ba Van) I> Ch, CX,MuP,TFI	Pam,GTh	27.05.15	-
164	-	Y	-	-	M	Poor	Ac iths-with sac Infec	Sac	Ul	Hewes Snappyvoget	P	Jnv>,GTh,.CIP,F,CB,XfM(Ba Van) I> Ch, CX,MuP,TFI	Car,Pam	27.05.15	-
	-	-	-	-	-	-	-	-	-	-	-	B,GTh,CIP,F,CB,Ba Van) I>XfM,MuP,Pam,Cir	Car	-	-
165	-	Y	-	-	F	Poor	Irregular	Prt	Ul	Sauvaus	S	Jnv, CIP,F,CB,.XfM,Ba Van) I> Ch,CX,MuP,TFI	Car,Pam,CIP,GTh,Ch	27.05.15	-
166	-	-	Y	-	F	Poor	Folliculitis	Prt	Ul	Sauvaus	S	GTh,CIP,F,CB,.XfM,Ba Van) I> Ch,CX,MuP,TFI	Jnv, Car,Pam	27.05.15	-
167	-	-	Y	-	F	Poor	Irregular Nodular	Prt	Ul, II	Sauvaus	S	Jnv, CIP,F,CB,.XfM,Ba Van) I> Ch,CX,MuP,TFI	Car,Pam,GTh	29.05.15	-

168	-	-	Y	-	M	Poor	Fallcutrits	Prt	ul, II	Sarwars	$	Arv; Car, CP, F, CB, ATM (Ba'vsn) > Ch, CX,MvP, TT	Part, GTh	29.06.15	-
169	-	Y	-	-	M	Poor	Fallcutrits	Prt	Sotis	Sarwars	$	Arv; CP, F, CB, Ba, ATM (Vsn) > CX, MvP, TT	GTh, Car, Part, Ch	29.06.15	-
170	-	Y	-	-	F	Poor	Jobless with no chi Pearl	Sac	ul	-	-	-	-	29.06.15	-
171	-	-	Y	-	F	Poor	Air bits with sac Infec	Sac	II	Sarwars	$	Car GTh, CP, Ba (vsn) > Ch, CX,MvP, TT	Arv, Part, F, CB, ATM	29.06.15	CONSTITUTIONAL RIGHTS ACT
172	-	-	-	Y	M	Poor	Fallcutrits	Prt	Sotis	Sarwars	$	Car GTh, CP, F, CB, ATM (Ba'vsn) > Ch, CX,MvP, TT	Arv, Part	29.06.15	-
173	Y	-	-	-	M	Poor	Impetigo	Prt	II	Sarwars	$	Arv; GTh, CP, F, CB, ATM (Ba'vsn) > Ch, CX,MvP, TT	Car,Part	30.06.15	-
174	-	Y	-	-	F	Poor	Impetigo	Prt	Chrisbella	Bonus Simp prospect	P	Arv; GTh, F, CB, ATM (Ba'vsn) > Ch, CX,MvP, TT	Car, Part, CP	30.06.15	-
	-	-	-	-	-	-	-	-	-	-	-	Part, B, GTh, CP, F, CB, Ba, Vsn) > MvP, Cr	Car	-	-
175	-	-	Y	-	F	Poor	Jobless with no chi Pearl	Sac	ul, II	Drewsa cancl	-	F, HG, CP, Ba'vsn) > TT	Arv, Part	30.06.15	-
176	-	Y	-	-	F	Poor	Fallcutrits	Prt	Chrisbella	Sarwars	$	Arv; GTh, CP, F, CB, ATM (Ba'vsn) > Ch, CX,MvP, TT	Part, Car	03.06.15	-
177	-	Y	-	-	F	Poor	Impetigo	Prt	II	Sarwars	$	Car, GTh, F, CB, Ba (vsn) > CX, MvP, TT	Arv> Part, Ch, ATM, CP	03.06.15	-
178	-	-	Y	M	F	Poor	Fallcutrits	Prt	ul	Sarwars	$	Arv; Car, GTh, CP, ATM (Ba'vsn) > Ch, CX,MvP, TT	Part, F, CB	03.06.15	CONSTITUTIONAL RIGHTS ACT
179	-	-	Y	-	F	Poor	Jobless with sac Infec	Sac	Sotis	Sarwars	$	Arv, CP, F, CB, ATM (Ba'vsn) > Ch, CX,MvP, TT	GTh, Car, Part	03.06.15	-
180	Y	-	-	-	F	Poor	Ecthma	Prt	II	Bonus Snapp prospect	P	Arv; GTh, CP, F, CB, ATM (Ba'vsn) > Ch, CX,MvP, TT	Car,Part	03.06.15	-
	-	-	-	-	-	-	-	-	-	-	-	Part, B, GTh, CP, F, CB, Ba, Vsn) > MvP, Cr	Car	-	-
181	-	-	-	Y	M	Poor	Jobless with no chi Pearl	Sac	ul	Sarwars	$	Car, CP, Ba'vsn) > Ch, MvP, TT	GTh, Part, CB, ATM, CX	05.06.15	CONSTITUTIONAL RIGHTS ACT
182	-	-	Y	-	M	Poor	Fallcutrits	Prt	ul	Sarwars	$	Arv; GTh, F, CB, ATM (Ba'vsn) > Ch, CX,MvP, TT	Car, Part, CP	05.06.15	-
183	-	-	Y	-	M	Poor	Impetigo	Prt	ul	Sarwars	$	Arv; Car, CP, F, CB, ATM, (Ba'vsn) > Ch, CX,MvP, TT	Part, GTh	05.06.15	-
184	-	Y	-	-	F	Poor	Impetigo	Prt	ul	Sarwars	$	Car GTh, CP, Ch, Vsn) > CX, MvP, TT	Arv; Part, ATM, BO, F, CB	05.06.15	INDUCIBLE CLINDAMYCIN RESISTANCE
185	Y	-	-	-	F	Poor	Pharyngotonsilitis Pearl	Sac	II	-	-	-	-	05.06.15	-
186	-	-	Y	-	F	Poor	Ecthma	Prt	ul	-	-	-	-	05.06.15	-
187	-	Y	-	-	M	Poor	Impetigo bullosa	Prt	Chrisbella	Sarwars	$	Car, CP, ATM, Ba'vsn) > Ch, CX,MvP, TT	Arv; Part, GTh, F, CB	05.06.15	CONSTITUTIONAL RIGHTS ACT
188	-	Y	-	-	F	Poor	Furunculosis	Prt	II	Sarwars	$	Arv; GTh, F, CB, Ba'vsn) > Ch, CX,MvP, TT	Part, Ch, CP, ATM, Car	05.06.15	-
189	-	-	Y	-	M	Poor	Air bits with sac Infec	Sac	ul, II	Sarwars	$	Arv; Car, F, CB, Vsn) > Ch, CX,MvP, TT	GTh, Part, ATM, BO, Ch, CB	05.06.15	CONSTITUTIONAL RIGHTS ACT
190	-	Y	-	-	M	Poor	Ir dermatitis with Inf	Sac	II	Bonus Snapp prospect	P	Arv; GTh, CP, CB, ATM (Ba'vsn) > Ch, CX,MvP, TT	Car, Part, F	05.06.15	-
	-	-	-	-	-	-	-	-	-	-	-	Part, B, GTh, CP, CB, Ba, Vsn) > MvP, Cr	Car	-	-
191	-	-	Y	-	M	Poor	Impetigo	Prt	II	Sarwars	$	Arv; GTh, CP, CB, ATM (Ba'vsn) > Ch, CX,MvP, TT	Part, Car, F	05.06.15	-
192	-	Y	-	-	F	Poor	Impetigo	Prt	Dyla	Sarwars	$	CP, F, CB, Ba'vsn) > CX,MvP, TT	Arv; GTh, Part, Ch, ATM, Car	05.06.15	-
193	Y	-	-	-	M	Poor	Jobless with no chi Pearl	Sac	ul	Sarwars	$	Arv; GTh, ATM, (Ba'vsn) > Ch, CX,MvP, TT	Part, F, CB, Car	05.06.15	INDUCIBLE CLINDAMYCIN RESISTANCE
194	-	Y	-	-	M	Poor	Ecthma	Prt	II	Smas invasense	$	Part, GTh, B, CP, F, CB, Ba, Vsn) > MvP, TT, Cr, ATM	Car	05.06.15	-
195	-	-	Y	-	M	Poor	Jobless with no chi Pearl	Sac	II	Sarwars	$	CP, F, CB, ATM, Ba'vsn) > Ch, MvP, TT	Arv; GTh, Car, Part, Ch	05.06.15	-
196	Y	-	-	-	Y	Poor	Impetigo	Prt	II	Sarwars	$	Arv; GTh, CP, F, CB, ATM, Ba'vsn) > Ch, CX,MvP, TT	Part, Car	17.06.15	-
197	-	Y	-	-	M	Mid Pus	Furunculosis	Prt	Chrisbella	Sarwars	$	CP, F, CB, ATM, Ba'vsn) > Ch, CX,MvP, TT	Part, Car, GTh, Arv	17.06.15	-
198	-	Y	-	-	F	Poor	Fallcutrits	Prt	II	Sarwars	$	GTh, F, CB, ATM, Ba'vsn) > CX,MvP, TT	Arv, Part, Car, CP, Ch	17.06.15	-
199	-	Y	-	-	M	Poor	Impetigo	Prt	II	Sarwars	$	Arv; CP, CB, ATM, Ba'vsn) > Ch, CX,MvP, TT	Part, Car, GTh, F	17.06.15	-
200	-	-	Y	-	M	Poor	Jobless with no chi Pearl	Sac	II	Sarwars	$	Arv; GTh, CP, F, CB, ATM, Ba'vsn) > Ch, CX,MvP, TT	Car,Part	17.06.15	-

KEY TO MASTER CHART

Pen	Penicillin
Amp	Ampicillin
Amx	Amoxicillin
GEN	Gentamicin
Cot	Co-trimoxazole
E	Erythromycin
CIP	Ciprofloxacin
CD	Clindamycin
AZM	Azithromycin
DO	Doxycycline
CX	Cefoxitin
CN	Cephalexin
CTR	Ceftriaxone
B	Bacitracin
LZ	Linezolid
VAN	Vancomycin
TEI	Teicoplanin
MUP	Mupirocin
M	Male
F	Female
S	Single(monomicrobial)
P	Polymicrobial
Pri	Primary
Sec	Secondary
UL	Upper limb
LL	Lower limb